FUNCTIONAL, AND DEGENERATE OR RUDIMENTARY GLOMERULI IN THE KIDNEY OF TWO SPECIES OF AUSTRALIAN FROG, CYCLORANA (CHIROLEPTES) PLATYCEPHALUS AND ALBOGUTTATUS (GÜNTHER)

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FOUR FIGURES

In an early paper concerned primarily with the openings of the nephrostomes from the coelom (peritoneal funnels), Sweet ('07) included a description of the general renal morphology of three species of burrowing, estivating Anura (Notaden bennetti, Heleioporus pictus, and Cyclorana [Chiroleptes] alboguttatus), all native to Australia.

In all three species the peritoneal canals, which open directly into the venous sinuses, were relatively numerous. The kidneys were remarkably and uniformly vascular, leading Sweet to state that the general appearance microscopically is that of a groundwork of blood corpuscles in which the tubules and Malpighian bodies were embedded. The number and size of the glomeruli present were not so constant. In Notaden the Malpighian bodies were described as being normal in number, round and somewhat small in comparison with the size of the kidney. In Cyclorana alboguttatus the glomeruli were also rounded but very few in number and remarkably small when compared with the size of the kidney. Sweet further states that in many sections no signs of Mal-

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pighian bodies were to be seen. In Helioporus the glomeruli were spherical and much more numerous than in Cyclorana alboguttatus.

Apparently the kidneys of these forms have not been reexamined and Sweet's work has not been generally quoted in the literature. Marshall and Smith ('30) cite Sweet's observations accurately as to the reduced size and number of glomeruli in Cyclorana alboguttatus but Baldwin ('48, p. 43), seemingly impressed by Sweet's statement regarding the absence of glomeruli from many microscopic sections of the kidney, referred to the condition in the desert frog, Chiroleptes, as aglomerular. It seemed of interest to reexamine the kidney of this species in which Sweet recorded reduction in both size and number of the glomeruli.

Two species of Cyclorana (Chiroleptes) were made available through the courtesy of Mr. Arthur Loveridge, Curator of Reptiles and Amphibians, Museum of Comparative Zoology. One specimen of Cyclorana platycephalus (male, anterior nares to anus, 62 mm) and two specimens of Cyclorana alboguttatus (females, anterior nares to anus, 56 and 62 mm respectively) were studied. The details of the histological condition of the tissues has been previously reported (Dawson, '48) and it is sufficient to state that the histological preservation of the kidneys was remarkably good. The specimen of Cyclorana platycephalus was collected in Western Australia. Specimen A of Cyclorana alboguttatus was collected in New South Wales. Specimen B came from Queensland Museum through exchange. The locality from which it was collected was not recorded. There were no records to indicate the time of year or the conditions under which the animals had been collected. Complete transverse, serial sections, 6 μ thick were made of all three kidneys. They were stained routinely, in hematoxylin and eosin.

DESCRIPTION

Sweet's observations on the high degree of vascularity of the kidneys in burrowing, estivating forms are fully confirmed

in this study of two species of Cyclorana. Fibrous connective tissue was reduced to a minimum both about the tubules and in the kidney capsule. The uriniferous tubules appeared to be suspended in a vascular bed, with the epithelium of the tubules separated from the large thin-walled venous sinuses by only a thin membrane. In specimen A of Cyclorana alboguttatus the sinuses were fully distended and gorged with hemolyzed erythrocytes (fig. 1). In specimen B the sinuses were collapsed and contained few blood cells. In platycephalus the sinuses were only moderately distended.

Ciliated peritoneal funnels were numerous and prominent and their entrance into the sinuses was diagrammatically clear in many instances. Sweet records a count of 210 funnels in a single kidney of alboguttatus which is comparable to the number (200–250) reported by Rugh ('38) in the common frog, Rana pipiens. The number of peritoneal funnels in Notaden bennetti reported by Sweet was remarkably high, 1067 in one kidney, but in another burrowing form Heleioporus pictus there were only 105 funnels in a kidney (Sweet, '07).

The topographical relationships of the uriniferous tubules of the kidneys of the two species of Cyclorana conform closely to the general anuran plan with the several segments of the tubules, as seen in cross section, segregated into three rather definite crescentic zones. A narrow ventral zone consisted almost entirely of distal convoluted segments and peritoneal funnels. The definitive proximal segments were uniformly restricted to a wide dorsal zone, with the glomeruli rather regularly spaced in a less definite intermediate zone (figs. 1 and 2).

Only near the medial border of the kidneys did the glomerular condition resemble that described by Sweet ('07). Here the glomeruli were small, sometimes irregular in form, sometimes rounded, with a scanty capillary supply but more laterally they were progressively larger and more elongated reaching maximum size and elongation in the lateral third of the organ. Since the axis of elongation passed through the vascu-

lar and free poles, the glomeruli of the lateral half of the kidney approximated a radial pattern, as viewed in cross section (fig. 2). The largest glomeruli of alboguttatus measured 120 μ on the long axis and 40 μ on the minor axis. In platycephalus the glomeruli were somewhat larger, attaining a maximum size of 180 μ on the long axis and 70 μ on the minor axis (fig. 4). In both species the small, more spherical glomeruli near the medial border ranged from 30 to 50 μ in diameter.

The medio-lateral gradation in size, shape, and degree of vascularization of the glomeruli of these two forms is difficult to explain. Without a knowledge of the early histogenesis of the kidney it is impossible to determine whether one is dealing with degenerate or rudimentary structures. In all instances in which the material was favorable for study the small glomeruli appeared to be associated with patent necks and proximal convoluted tubules. However, in the extreme medial borders small, coiled, basophilic cords without glomeruli were seen. They may represent either degenerated uriniferous tubules or vestiges of peritoneal canals.

It is interesting to note that the best developed glomeruli were those farthest from the dorsal aorta. The renal arteries entered the kidney on the ventral surface near the medial edge; some arterioles passed directly to the more medial glomeruli, others, progressively longer, ran near the ventral border of the kidney as almost straight vessels to curve gently dorsally to enter the lower poles of the glomeruli.

The intracapsular spaces especially of the larger Malpighian bodies were frequently greatly distended and filled with coagulum (fig. 3) evidences, according to Grafflin ('33) in his studies of glomerular degeneration in the kidney of the daddy sculpin, of constriction of the neck segments. However, in the Cyclorana kidney the lumina of the necks were as large as those of the proximal convoluted segments which they join, and coagulum was also usually present in the lumina of the convoluted tubules.

The epithelium of the necks was always low and flattened and the nuclei were also small, flattened and pyknotic (fig. 3).

No cilia were present. The proximal and distal convoluted tubules and collecting tubules appeared normal, with the exception of the nuclear patterns of the proximal segments previously described (Dawson, '48).

The narrow ciliated intermediate segment, which is typical of anuran kidneys, could not be recognized. In a few instances there appeared to be very short non-ciliated segments, in which neither a brush border nor vertical striation was evident. In most cases there was an abrupt transition from proximal to distal convoluted epithelium.

DISCUSSION

It is difficult to harmonize these observations on the glomeruli with those of Sweet. While Sweet does not specifically state that she was studying serial and transverse sections, the implications in other parts of her descriptions are that she was doing so. Random, longitudinal sections could readily present the picture she described. It is possible, but rather unlikely, that the glomerular condition would vary seasonally depending on whether the animals were taken when water was plentiful or during periods of estivation.

While no attempt has been made to count accurately the number of glomeruli present in a single kidney, rough calculations indicate that there are over one thousand, with from 40 to 60 per cent of normal or near normal size. Thus the kidneys of Cyclorana are not aglomerular but many tubules near the medial border approach very nearly to this condition.

As Marshall and Smith ('30) point out a large amount of work has been done on the kidneys of Amphibia but with the exception of these estivating forms there is no evidence of any considerable glomerular reduction. The most constant feature of all the burrowing Anura, that have been studied, is the high degree of vascularization of the kidneys. The peritoneal funnels are always numerous but are not uniformly present in abnormally high numbers. In Notaden bennetti where the peritoneal funnels may number over a thousand the

glomeruli are normal in number, rounded and small in relation to the size of the kidney.

During the wet season the burrowing estivating Anura of Australia possess remarkable powers of storing water especially in the urinary bladder, subcutaneous tissues, and peritoneal cavity. They imbibe water chiefly through the skin and mouth. The intake is apparently very rapid. A correspondent of Buxton ('23) writes that "if you put a lean, dry, herring-gutted Chiroleptes into a beaker with two inches of water, in two minutes your frog resembles a somewhat knobby tennis ball." The fully distended animal is occasionally used by the Australian aborigine as a source of drinking water.

In the dry season these animals remain in permanent burrows about a foot below the surface of the soil which is baked into a hard mass of clay by the sun. Water loss under these conditions will be reduced to a minimum and the metabolic activity of the animal will also be low. The early postulation of Sweet ('07) of the maintenance of a constant recirculation of stored water for general body needs seems valid. Water filtered off by the glomeruli would enter the urinary bladder, thence to the body cavity and then pass by way of the ciliated peritoneal funnels into the venous drainage to be again returned to the glomeruli. Under these conditions a reduction of glomerular filtration would seem to have some adaptive value during estivation since it would tend to diminish the rate of recirculation of water. Presumably in the inactive state of estivation the concentration of wastes in the body fluids would not reach a toxic level.

The effectiveness of the ciliated funnels in draining fluid from the peritoneal cavity must be emphasized, although similar functioning canals are present in many Anura which have free and continuous access to water. In Necturus, in which the peritoneal canals open directly into primary uriniferous tubules the cilia of the funnel can exert a pressure equal to 8 to 11 cm of water, while those of the tubule neck develop a maxium pressure of 4.0 to 5.7 cm of water (White,

'29). The rate of clearance from the body cavity in Rana pipiens, where the peritoneal funnels open into venous sinuses, has been studied by Rugh ('38). He found that within an hour, following intraperitoneal injections of a solution of India ink, the renal vessels were blackened with ink particles. Ink was also found in the inferior vena cava and in the liver lobes. After 5 hours there was little ink in the body cavity; the kidneys, liver, heart and lungs were blackened by ink within their vessels. Even the arteries of the testes contained ink.

There is also evidence in Necturus of physiological constriction of the ciliated neck and of contraction of the ciliated junctional segment between proximal and distal tubules of the frog (Lucas and White, '33; Singer, '33). The physiological significance of these contractile mechanisms in regulating intratubular flow is a matter of conjecture. Presumably the intratubular pressure and rate of fluid passage in Cyclorana would tend to be considerably reduced by the absence of cilia from the epithelium of the neck and the virtual absence of an intermediate, ciliated segment, a condition probably favorable for tubular reabsorption of water.

SUMMARY

The kidneys of two species of Australian desert frog, Cyclorana alboguttatus (two specimens) and platycephalus (one specimen) were found to exhibit wide variations in the size and appearance of the glomeruli. Near the medial border the glomeruli were small, often irregular in form but sometimes rounded. More laterally they were progressively larger and more elongated, reaching maximum size and vascularization in the lateral one-third of the organ. Whether the mediolateral gradation in size, shape and degree of vascularity was due to developmental failure or represented secondary, regressive changes, perhaps influenced by estivation, could not be determined.

The necks of the tubules lacked cilia and their epithelial cells were greatly flattened with small pyknotic nuclei. In ad-

dition the intermediate segment typically ciliated in Amphibia was absent or represented only by a few non-ciliated cells, interposed between proximal and distal convoluted segments. Otherwise the tubular differentiation conformed closely to the general anuran plan.

Ciliated peritoneal funnels, emptying directly into venous sinuses, were present, but did not occur in abnormally high numbers. The renal sinuses were extremely large and numerous, the peritubular connective tissue being reduced to a minimum. The kidneys of Cyclorana are not aglomerular but many tubules located near their medial borders closely approach this condition.

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PLATES

DESCRIPTION OF PLATES

All figures are photomicrographs made from complete, transverse, serial sections of kidneys of Cyclorana alboguttatus and Cyclorana platycephalus, cut 6 μ thick, and stained in hematoxylin and eosin.

PLATE 1

EXPLANATION OF FIGURES

1 Low power view of a transverse section of an entire kidney from Cyclorana alboguttatus, specimen A, medial border to the left, dorsal side at the top, showing the general pattern of distribution of the glomeruli and uriniferous tubules. The glomeruli, appearing as dark bodies, 14 in number, are smallest near the medial border and gradually increase in size to reach maximum volume in the lateral one-third of the organ. There is little interstitial connective tissue and the tubules are intimately bathed by a rich network of venous sinusoids, filled with hemolyzed erythrocytes. × 35.

2 A smaller area from the lower right portion of the kidney illustrated in figure 1, showing 5 well-developed glomeruli. Distal convoluted segments are seen at the left and proximal convoluted segments at the right of the photograph. Long, straight afferent arterioles enter the poles of the third and 5th glomeruli. Small islands of hemopoietic tissue are dispersed between the distal convoluted segments (upper left). × 125.

1

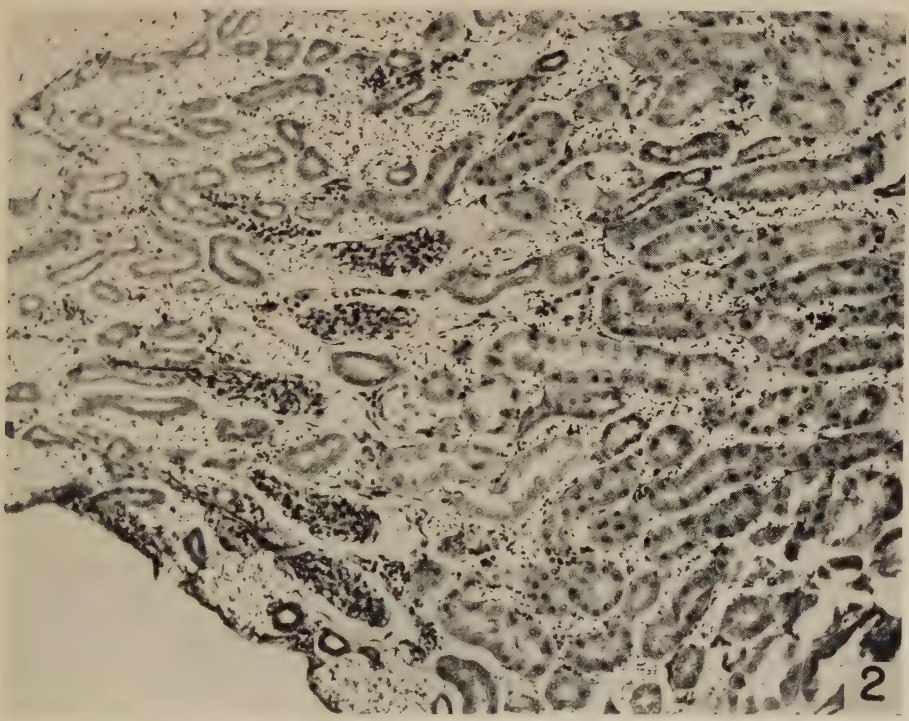
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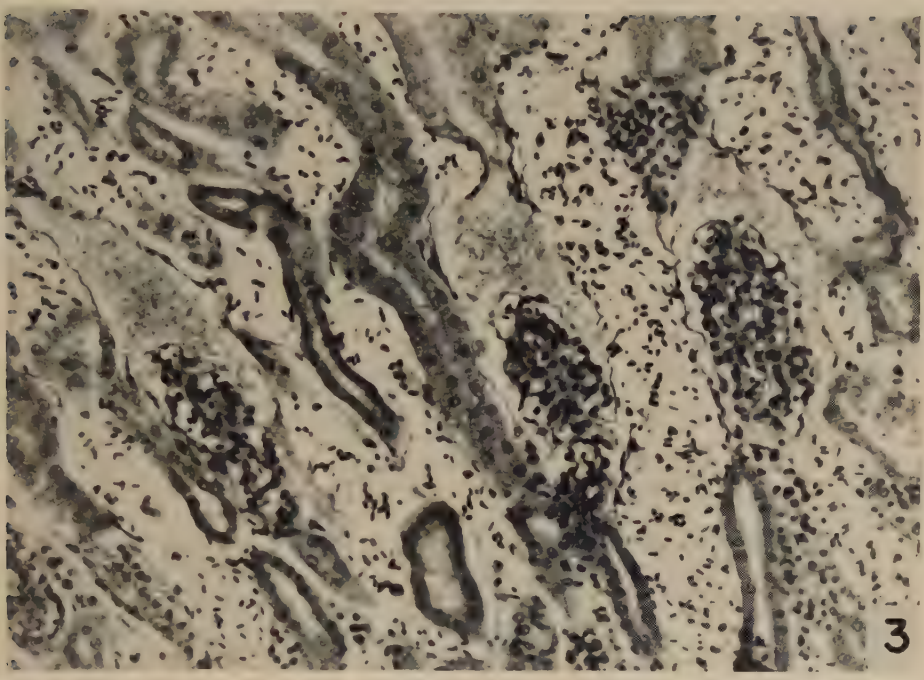
PLATE 2

EXPLANATION OF FIGURES

3 An area from the center of a transverse section of the kidney of Cyclorana alboguttatus, specimen A, with the same orientation as in figure 1, showing three glomeruli and the distended intracapsular spaces. The parietal wall of the capsule of the middle glomerulus is continuous with a short neck which joins a proximal convoluted segment dorsally. The epithelium of the neck is low and flattened with small densely stained nuclei. It is not ciliated. × 250.

4 An area from the kidney of Cyclorana platycephalus showing a richly vascularized glomerulus which is somewhat larger than comparable glomeruli of alboguttatus. A straight afferent arteriole is seen entering the attached pole of the glomerulus and a curved efferent vessel leaving to enter a venous sinus. Numerous islands of hemopoietic tissue lie between the walls of the tubules and the venous sinuses. × 250.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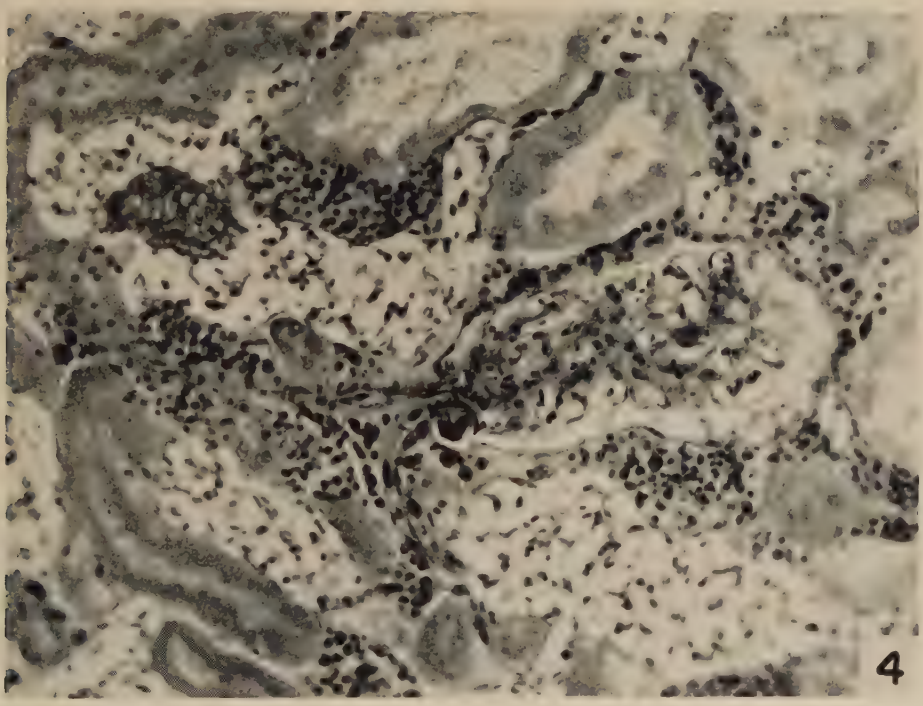
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OVARIAN CHANGES IN IMMATURE RABBITS INDUCED BY EQUINE GONADOTROPHIN

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TEN FIGURES

In a study of ovaries of sexually immature macaques, pregnant mare serum (PMS) was observed to produce polyovular follicles within cystic ovaries (Lloyd and Rubenstein, '41). The present study was initiated in an effort to determine the origin of these polyovular follicles with particular interest in the entire process of ovogenesis as it may be influenced by this gonadotrophin. Colchicine, an alkaloid, arrests mitotic divisions in metaphase and, therefore, accentuates any trophic response produced by a gonadotrophin. The growth effect of theelin upon the genital tissues of mice was demonstrated by use of colchicine by Allen, Smith and Gardner ('37). Schmidt and Hoffman ('41) employed the colchicine technique in observing proliferation and ovogenesis in germinal epithelium of the normal mature guinea pig ovary. A study of differential growth in ovaries of rats treated with colchicine under the stimulation of the hormones at the time of ovulation was also reported by Allen, Thomas, Wilson, and Hesson ('43).

In our rabbits colchicine was injected following a course of treatment with pregnant mare serum. Mitotic counts were made on serial sections of ovaries from experimental and control animals. Observations reported relate the presence of multiovular follicles to the generalized overgrowth of the epithelial elements in the ovary.

MATERIALS AND METHODS

Pregnant mare serum [1] was injected into 8 of 12 immature female rabbits, divided into two groups of 4 each. One group was given a small quantity of gonadotrophin over a long period; the other a larger quantity over a short period. The remaining 4 animals served as controls. Group I (A, B, C, D) of the experimental rabbits received 5 rat units of the equine gonadotrophin once a week for one month for a total of 20 units. Group II (G, H, I, J) received 10 rat units every other day for one week for a total of 30 units. Of the remaining 4, two were injected with normal saline (E, F), one was completely untreated (L) and the 4th died before the termination of the experiment.

Colchicine salicylate in saline was injected into 6 of the 8 experimental animals and two of the three surviving controls, 24 and 18 hours before ovariectomy.

The ovaries were removed and fixed in Zenker formol. Serial sections were prepared and stained with iron hematoxylin and eosin. The histological sections were studied and mitotic counts were made of every 10th section of one ovary from each animal. Within every 10th section only follicles containing ova were examined for mitotic figures.

HISTOLOGICAL OBSERVATIONS

Group I

In response to the small quantity of pregnant mare serum injected over the longer period, the ovaries of experimental rabbits, A, B, C, and D showed many changes not demonstrated by control rabbits E and F (figs. 3 and 4). The conversion of the ordinarily single layered germinal epithelium into a many layered structure in many areas and particularly at the end of the ovary nearest the Fallopian tube was observed (fig. 5). The proliferated germinal epithelium frequently invaginated below the surface forming rosettes or "Pfluger's" tubes (figs. 5 and 6). Polyovular follicles (fig. 9) were

[1] Gonadogen, Upjohn Co., commercial preparation used.

present in the ovaries of all the experimental animals of this group. The granulosa cells of the medium-sized follicles showed many mitotic configurations in evidence of great proliferation in the follicular elements. The thecal cells exhibited but little sign of undergoing an increased rate of growth. However, the blood vessels of the theca interna were greatly enlarged and distended as they were elsewhere in these ovaries. Under the influence of the gonadotrophin the ovaries appeared to be crowded with ova and various sized normal follicles but no follicles showed identifiable cystic degeneration. No corpora lutea were present. Mitotic divisions were not found among the ova.

Group II

The ovarian response of experimental rabbits G, H, I, and J to the larger dose of gonadotrophin injected within the brief span of one week differed in several ways from the effects observed in the animals of Group I. Proliferation of the germinal epithelium was present but with less tendency to invaginate below the surface. Rather hillocks of cells tended to form above the surface (fig. 7). Mitotic divisions were prevalent in the germinal epithelium in these animals. Polyovular follicles occurred in ovaries of two of the 4 animals (G and J) while H and I showed double follicles and medium-sized follicles possessing primary follicles within their thecae (fig. 10). The granulosa of medium-sized ovarian follicles from animals of Group II also showed an increased rate of growth in the presence of many cells in mitotic division (fig. 8). Thecal cells exhibited similar evidence of stimulation. However, in these ovaries many follicles had swollen with fluid until a "window-pane" effect was produced. Since these very large follicles were found to possess but a thin layer of granulosa cells among which were few if any dividing cells but much nuclear fragmentation; their extreme size was attributed to cystic degeneration rather than to overgrowth. The blood vessels had become extremely distended, engorged

TABLE 1

Mitotic count of ovarian sections[1]

ANIMAL	PROCEDURE	GRANULOSA CELLS		THECA CELLS		GERMINAL EPITHELIUM CELLS
		Total number	Number in middle 10 sections	Total number	Number in middle 10 sections	Total number
Group I						
A	PMS — 20RU/1 mo. + colchicine	228	108	9	6	12
B	PMS — 20RU/1 mo. + colchicine	300	180	17	17	5
C	PMS — 20RU/1 mo. + colchicine	171	119	28	23	3
D	PMS — 20RU/1 mo.	58	51	18	16	7
E	Control — Colchicine only	20	20	0	0	0
F	Control — Colchicine only	36	36	4	4	0
Group II						
G	PMS — 30RU/1 wk. + colchicine	840	465	399	160	17
H	PMS — 30RU/1 wk. + colchicine	3149	1062	477	191	99
I	PMS — 30RU/1 wk. + colchicine	1245	1035	563	387	43
J	PMS — 30RU/1 wk.	338	156	163	64	4
L	Control — Untreated	37	23	2	2	0

[1] The mitotic figures were counted in the follicles containing an ovum (or ova) in every tenth section of an ovary from each rabbit.

with blood, and in many instances, had ruptured into the antrum. No corpora lutea or mitotic configurations within the ova were observed.

INCIDENCE OF MITOSES

The degree of proliferation occurring in the ovarian tissues was determined by actual count of mitotic divisions among granulosa cells, cells of the thecae and the germinal epithelium

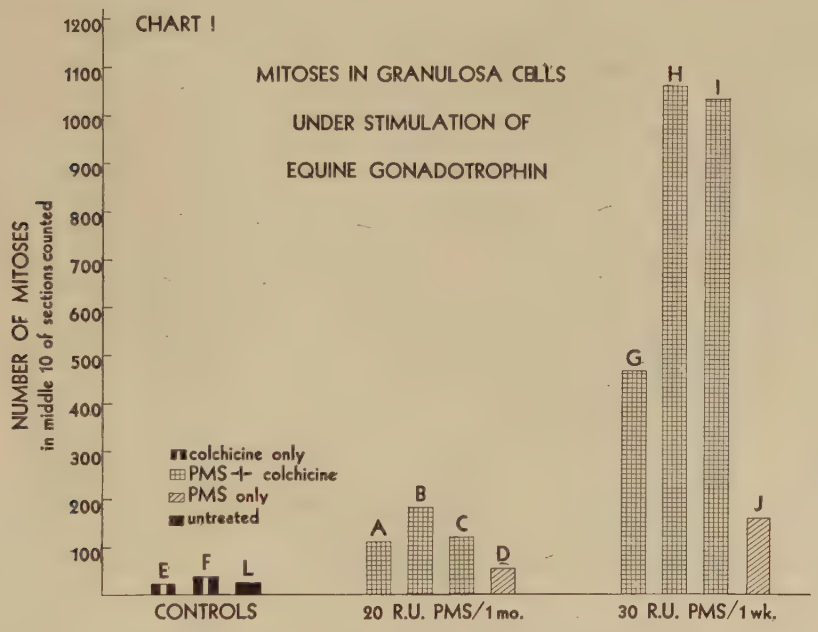

Fig. 1 Increased rate of cell division of granulosa cells in ovaries from PMS treated rabbits, and augmentation of this response by colchicine.

(table 1). Proliferation of the granulosa in response to gonadotrophin occurred in all of the animals receiving the pregnant mare serum (PMS) whether in large or small doses, i.e., the experimental rabbits of Group I and II. The mitotic count was greatly increased in the animals receiving colchicine as well as equine gonadotrophin. However, the rate of cell division was so low in the untreated rabbits which received

only colchicine that an accentuation by mitotic arrest produced but a minimal count. Where the quantity of gonadotrophin injected was great in proportion to the size of the animal (Group II), the ovarian response showed excessive follicular development, and the mitotic count of granulosa cells was high (fig. 1). The mitotic count for the thecal cells showed an

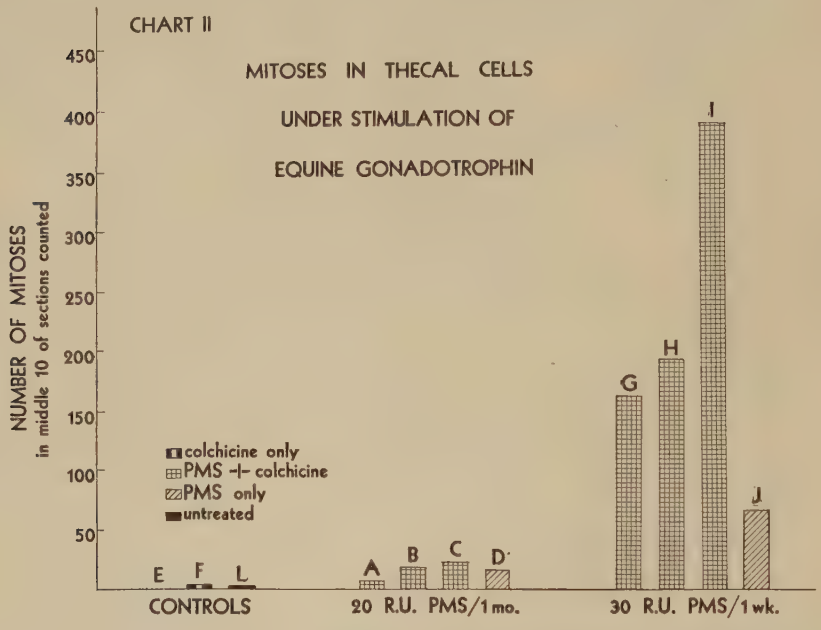

Fig. 2 Increased mitotic count among thecal cells in response to PMS accentuated by colchicine.

increase in all PMS treated animals and the rise was augmented by the larger injection (fig. 2). No mitoses were observed in the interstitial tissue, which is fairly abundant in both experimental and control animals, as is characteristic of the rabbit ovary.

DISCUSSION

Since the biological properties of the gonadotrophins, whether pituitary or chorionic in origin, have long been known, the proliferative influence of these substances upon

ovarian tissue is an established fact. It was sought here to determine whether all of the cells are equally affected by the stimulatory action. In this study proliferation of the germinal epithelium was observed in the ovaries from animals both receiving a small dosage of gonadotrophin over a long period and a larger dosage over a short period. The germinal epithelium of the ovaries from these animals exhibited areas of growth in which the single layer of cuboidal cells characteristic of germinal epithelium had become a many layered tissue with hillocks projecting from the surface and invaginations producing tubules of epithelial cells just below the surface. The cross sections of these tubules have been referred to in the observations as "rosettes" of epithelial cells. Mitotic configurations occurred more frequently in the ovarian germinal covering in rabbits receiving large doses of PMS for a short period (Group II), than in rabbits receiving small doses of the gonadotrophin for a longer period (Group I). However, there were few mitotic figures in the epithelial covering in both groups, considering the extent of growth and the number of such figures present among the granulosa cells of the follicles.

The cyclic formation of new ova in the mature mouse by means of proliferation of the germinal epithelium has been found to reach a maximum at the time of oestrus when the genital organs are hyperemic (Allen, '23). Papanicolaou ('24) described oogenesis in the guinea pig as a continuous process with spurts due to various nutritive and hyperemic conditions. Dilatation and engorgement of the ovarian blood vessels was observed as a constant response to the gonadotrophin. It is also of interest that the germinal epithelium had several layers of cells in ovaries of a 15 mm human embryo and a 43 mm fetus (Simkins, '28). A thickening of the germinal epithelium in a pregnant dog was recorded by Wagener (1879), while Koster (1868) described epithelial invaginations in the ovaries of recently pregnant women, aged 32 and 37 years, and in young girls of 16 and 17 years.

The classic study of Evans and Swezy ('31) did much to establish further the fact that ovogenesis in mammals is a cyclic process. They interpreted proliferations of new ova from the germinal epithelium in the form of invaginations or cords as a part of the rhythmic production and destruction of tissue within the generative tract. The rhythm of this cycle was found not to be obliterated by pregnancy. Schmidt and Hoffman ('41) demonstrated on sexually mature guinea pigs injected with colchicine that the mitotic rate in the germinal epithelium was much higher during the estrous period than at any other time in the sexual cycle. Latta and Pederson ('44) traced the formation of new ova from the germinal epithelium in young rats by injecting intravital dye beneath the periovarian membrane characteristic of this species. Their observations of a thickening of the germinal epithelium into two to three layers of cells, and of invaginations of cords and crypts of germinal epithelial cells with small groups of enlarged, lightly stained cells containing dye inclusions at the end of these ingrowths would lend additional evidence that such proliferative changes are normal phenomena. Therefore, it might be concluded that the presence of growth and invagination of the germinal epithelium in ovaries of immature rabbits treated with a gonadothrophin (PMS) indicated merely an accentuation of the normal cyclic pattern of proliferation.

The counts of mitotic divisions among the granulosa cells of Graafian follicles within the sectional ovaries in this study demonstrated (figs. 1 and 2) that not only were increased numbers of mitoses observed among these cells in response to the equine gonadotrophin but that the larger dose of the hormone greatly magnified the response. Although colchicine augments the action of the gonadotrophin, recorded by the number of mitoses arrested in metaphase, McPhail and Wilbur ('44) showed in a study upon rabbits that colchicine does not enhance the physiological action of the hormone.

When the ovaries were stimulated rapidly with heavy doses of pregnant mare serum the Graafian follicles were observed

to swell into cystic proportions. This phenomenon has been previously reported for the macaque by Van Wagener and Morse ('38) and Lloyd and Rubenstein ('41). This swelling of the follicles could not be attributed to a rapid multiplication of the granulosa cells, since it was observed here and by Schmidt ('42) that mitotic configurations occurred in greatest concentration in the granulosa and thecal cells of medium-sized follicles. The distended and engorged condition of the blood vessels of the cystic follicles in conjunction with the degenerative changes within the granulosa of these follicles would seem to support the observations of Reynolds ('47) on changes in the ovarian vessels under gonadotrophic stimulation. Reynolds ('47) reported that gonadotrophic stimulation caused the coiled ovarian arteries to become uncoiled and undulating unless completely disrupted by the development of cystic follicles due to too great a local pressure of blood during the growth of the follicle.

The presence of polyovular follicles within the ovaries of animals treated with pregnant mare serum is an observation which might have been anticipated on the basis of Hartman's ('26) conclusions as to the origin of such structures in correlation with evidence already presented here of increased growth in the epithelial elements. In this comprehensive report on polynuclear ova and polyovular follicles in the opossum and other mammals, Hartman attributes their presence to the excessive formation of germ cells in proportion to stroma with the result that this crowding causes more than one ova to become incorporated into a single follicle. Mainland ('28) in his study of pluriovular follicles in the ferret confirmed Hartman's conclusions.

The evidence here presented would further substantiate the concept that polyovular follicles are caused by over-production of ova by proliferation of the germinal epithelium, and overcrowding of rapidly developing follicles. No mitotic figures were observed within the ova. This failure to observe mitosis within the ova in PMS and colchicine treated animals would discount the possibility of formation of new ova from

previously existing ova, as suggested by Lloyd and Rubenstein ('41).

Multiple ova were not found within the large or cystic follicles in any of the ovaries. This might seem to imply that multiplicity of ova within a follicle was just a phase of the process of degeneration in which the ovum became fragmented. Such an explanation of multiple ova within medium-sized follicles in the cat was offered by Strassman ('36). However, in the multiple ova observed in the present study, the nucleus and the cytoplasm of both eggs were in excellent condition although, of course, the section showing the nucleus of one need not show the the nucleus of the other ovum. The granulosa around the multiple ova also appeared to be composed of normal, healthy cells although many atretic follicles showing fragmentation of the chromatic material within the granulosa cells and degeneration of the zona pellucida were observed in the same section. The fate of these multiovular follicles is undetermined. Wieman and Weichert ('36) reported on a twin pregnancy with but a single corpus luteum obtained at autopsy from a 25-year-old woman. They concluded that the embryos were "biovular in origin and that the two ova came from a single follicle."

SUMMARY

Proliferative changes in the ovaries of immature rabbits induced by equine gonadotrophin were investigated by the use of colchicine which arrests mitoses in metaphase. Histological observations and mitotic counts on serial sectons of ovaries from animals injected with pregnant mare serum (PMS) revealed a proliferation of epithelial elements with a correlation between the quantity of gonadotrophin administered and the extent of the trophic response. Pregnant mare serum injections of low dosage over a period of one month induced growth of the germinal epithelium into layers with invaginations below the surface as tubules; while heavy doses of PMS injected within a short period induced greater stratification of the germinal ovarian coverings with little tubular formation.

In response to pregnant mare serum the granulosa cells of medium-sized follicles from experimental ovaries showed an increased rate of division accompanied by a less extensive proliferation of thecal cells. Ovaries influenced by large doses of gonadotrophin within a week, gave evidence of a greatly augmented rate of division among the cells of medum-sized follicles as well as more mitoses among the germinal epithelial cells.

Polyovular follicles observed in ovaries from rabbits injected with pregnant mare serum were attributed to hypertrophy of epithelial elements and crowding of the innumerable ova thereby produced. The absence of mitotic figures in identified ova indicated new ova were not formed from previously existing ova. Neither corpora lutea nor evidences of follicular luteinization were demonstrable.

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PLATE 1

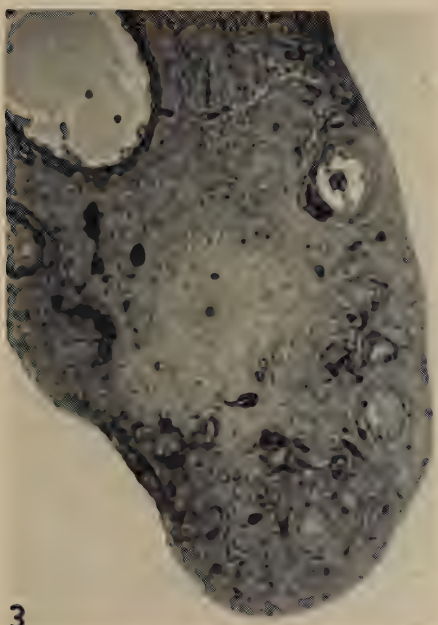
EXPLANATION OF FIGURES

3 Low power magnification (× 64) of ovarian section from control rabbit showing few follicles and ova within unstimulated ovary.

4 Low power magnification (× 64) of ovarian section from experimental rabbit demonstrating crowding of normal medium-sized follicles, ova and atretic follicles within ovary under stimulation of PMS.

5 Proliferation of germinal epithelium with "rosettes" of epithelial cells below surface in ovarian section from rabbit of Group I injected with pregnancy mare serum. (× 160).

6 Invagination of germinal epithelium of ovary as "Pfluger's" tube under stimulation of PMS. Cross sections of similar structures show the "rosette" effect seen in figure 5. (× 675).



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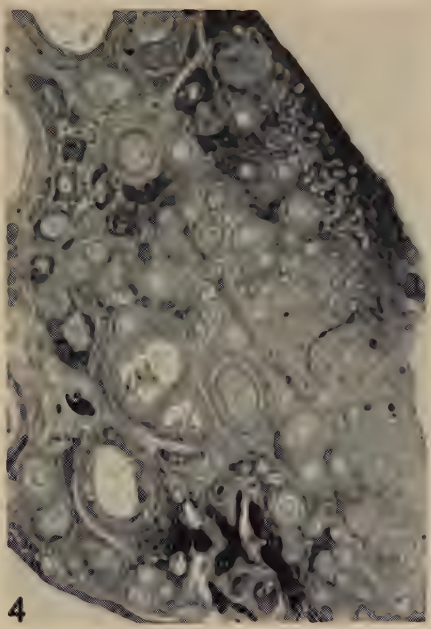

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PLATE 2

EXPLANATION OF FIGURES

7 High power magnification (× 300) of proliferation of germinal epithelium in ovarian section from rabbit H of Group II injected with PMS.

8 High power magnification (× 675) illustrating mitotic figures within granulosa and thecal cells of ovarian follicles in response to PMS and colchicine injections.

9 Polyovular follicles present in rabbit ovary following PMS injections. (× 160).

10 Graafian follicle developing within the thecal layers of larger follicle in ovary of PMS treated rabbit. (× 160).

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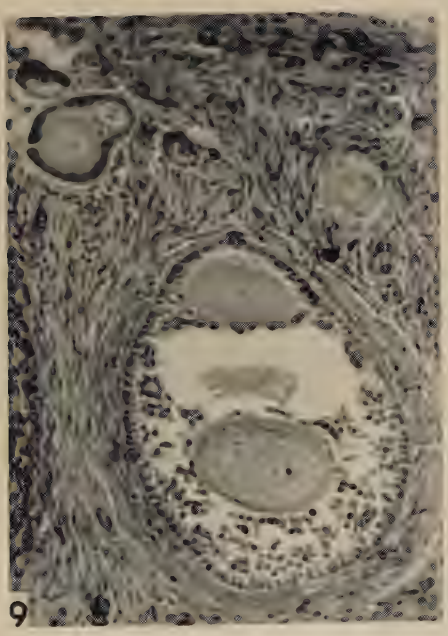
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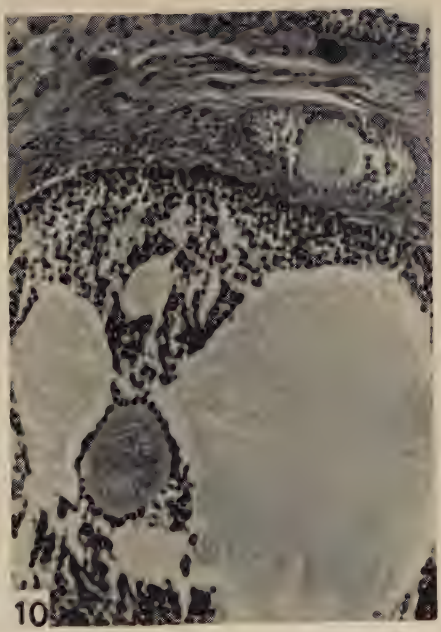
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THE INTERMEDIARY CIRCULATION IN THE RED PULP OF THE MOUSE SPLEEN [1]

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TWENTY FIGURES

The morphological characteristics of the arteries and the veins of the mammalian spleen are well known. Except for differences in terminology, differences probably due to variations in classes or species of the animals studied, and differing descriptions which may be explained partly by the variable constriction or dilatation of the spleen before its preparation for examination, most investigators are in substantial agreement concerning the arterial vascular tree down to the arterial capillary, and from the venous sinuses, or at least the venules, on outward to the large veins. Concerning the intermediary vascular pattern between the arterial capillary and the venous sinus, however, conflicting opinions have appeared in the literature for the past 90 years. Investigations of the connections between the arterial and venous channels of the splenic circulation, unlike the capillary beds of most other organs, have resulted in descriptions so contradictory that no consensus has been reached to the present day. No survey of the literature will be attempted in this report; the reader can find admirably adequate reviews in the monographs

[1] The studies, upon which this report is based, were supported during 1939–42 by a grant from the John and Mary R. Markle Fund, and during 1948–50 by a grant from the Life Insurance Fund.

Some of the observations here reported were submitted in abstract form in partial fulfillment of the requirements for a Master of Science degree granted the senior author in 1946.

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of Hartmann ('30), McNee ('31), Kyes ('32), Klemperer ('38), and Björkman ('47).

The controversy concerning the intermediary splenic circulation has been caused by the great difficulty of demonstrating the arteriovenous connections satisfactorily for microscopic study; injection studies, as well as those by the usual sectioning and staining methods, have failed to trace the blood flow from artery to vein with the same clarity as investigators have enjoyed in the study of most other organs.

A majority of investigators have concluded that the blood circulation through the spleen is "open," that the endothelium of the arterial capillary ends abruptly, and the blood then flows through the reticular meshes of the red pulp, not contained by endothelial-lined vessels, but flowing freely among the loose cells of the pulp. These writers conceive further of the venous sinuses as beginning in the red pulp as open funnels, or as an anastomosing network. The blood which lies free in the pulp is then forced into the sinuses, either into their open funnel-like origins or through their mural stomata, if they form a closed network, or by both pathways, from the pressure (tissue and hydrostatic) exerted upon the blood by contraction of the spleen.

A minority of investigators, however, have stated that they could demonstrate preformed, lined, intact channels connecting the arteries with the venous sinuses or venules. A few writers, finding evidence for both views in their preparations, have postulated that the spleen has simultaneously an "open" and a "closed" circulation, or a functionally "closed" circulation in a contracted spleen, with an "open" circulation in a dilated spleen.

A fact that has added fuel to the controversy, and that has been recognized even by the adherents of the "closed" circulation theory, is the constant presence of red blood cells in the extravascular meshes of the red pulp. If the vascular channels are continuous, it has been difficult to explain the extravascular location of varying numbers of red blood cells.

If the circulation is an open one, on the other hand, with the arterial capillaries opening freely into the pulp spaces, it is equally difficult to comprehend why the red blood cells are not always in concentrations equal to that in the circulating blood, or as we see them in regions of hemorrhage. Similarly, injected materials are found immediately free in the pulp spaces, whether they are large, as foreign nucleated red blood cells, or small, such as dye particles.

The mystery has been augmented, rather than lessened, by the fact that most writers, even those who speak of a "closed" circulation, have agreed on the essential structure of the venous sinuses, with their rod-like "barrel stave" endothelial cells longitudinally arranged, not contiguous, and surrounded by rings or spirals of reticular fibers, reminiscent of barrel hoops. This universally accepted concept brings up the question of the size and function of the mural stomata. Are the spaces between the endothelial cells small, so that injected particles and occasional red cells escape from the blood stream into the pulp spaces, as the "closed" circulation proponents believe? Or are the openings always large, so that red cells and leucocytes can be forced through them from the pulp spaces into the venous system, as the adherents of the "open" circulation theory suggest?

This dilemma on the intermediary vascular pattern of the spleen appeared to have been resolved in great part when Knisely ('36b) described the circulation as seen in the living mammalian spleen with the aid of a fused-quartz rod transillumination method devised by him (Knisely, '36a, '37, '38). He stated that the splenic vascular system consists entirely of preformed, interconnected, lined channels, a statement which would seem at first thought to agree with the earlier descriptions of a "closed" circulation. Knisely, however, deplored the use of the terms "closed" and "open" as inadequate to designate the circulation in the living spleen, because the venous sinuses concentrate the blood cells by leaking "blood fluid" to the extravascular spaces of the red pulp. He stated that occasional red blood cells leave the

lined vascular system by diapedesis or individual penetration of the intact vessel wall. As they pass through the wall, they are first distorted and then snapped forward suddenly. Recently Knisely ('50) has spoken of the "separatory" circulation of the spleen.

Knisely described the relations and activities of the venous sinuses, relating how they separate the blood cells from the fluid of the blood, either by a continuous filtration process, or by what he called a filtering-filling process. In the latter process the sinuses fill with blood, leaking nearly all the fluid to the surrounding extravascular pulp spaces, and packing the cells closely together. The cells are then stored in the sinuses for various periods of time before being released to the venules. Knisely also described long slender capillaries which by-pass the sinuses, and conduct blood from arterioles to venules when the blood cells are being stored in the sinuses. He suggested that these "shunts" nourish the splenic tissue, when the blood flow through the venous sinus route is at a stand-still.

Knisely stated that the various cycles of blood cell storage in the venous sinuses, their filling and emptying, their filtering of cells from fluid, or the rapid conduction of blood from arterial capillary to venule — all these varied activities are precisely controlled by sphincters located at strategic points along the vascular system, in the arteries, arterial capillaries, and at both the afferent and efferent ends of the sinuses. Figure 1, which is a modification of Knisely's figure 3 (Knisely, '36b), summarizes in diagrammatic form the main points of Knisely's report, showing the various types of venous sinuses in different stages of dilation.

After Knisely's paper appeared, David W. MacKenzie, at that time a member of the surgical department of Dr. Allen O. Whipple, Columbia University, spent three months in Knisely's laboratory, learning the fused-quartz rod transillumination technic. During this visit, Knisely also showed MacKenzie his method of studying the splenic circulation, and presumably demonstrated many of his reported observa-

tions. Subsequently, MacKenzie returned to Columbia, and continued the study of the splenic circulation for several years.

In 1941, MacKenzie, Whipple, and Wintersteiner ('41) published their investigations on the circulation in the living mammalian spleen, as studied by the transillumination technic. They denied essentially all of Knisely's findings, stating that the intermediary splenic circulation is of the "open" type,

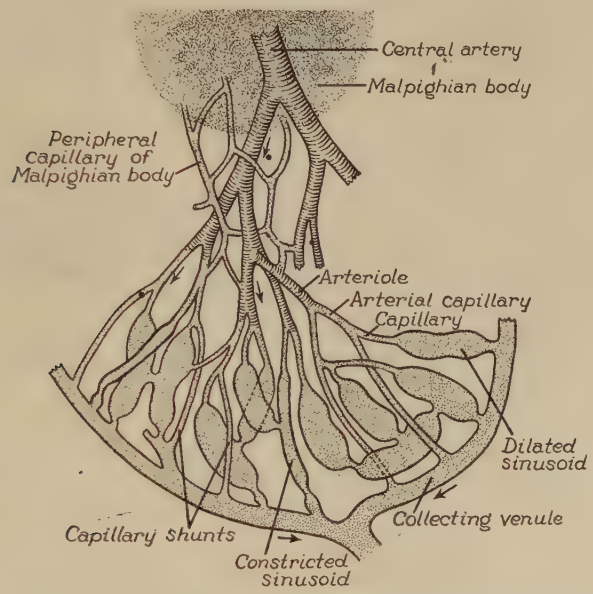

Fig. 1 Diagram of splenic circulation, modified from figure 3 of Knisely ('36b).

that there are no preformed, intactly walled connections between the arterial and venous systems.

MacKenzie and his coworkers found no evidence of the cyclical storage activities of the venous sinuses described by Knisely. According to them, the circulation through the spleen is more or less continuous, except for an occasional temporary blockage of a channel by a white blood cell, acting like a stopper in a bottle.

The connections between the arterial and venous systems, these authors stated, are pulp channels, the open interstices in the red pulp. They describe the arterial capillaries as terminating in the pulp spaces by a funnel-shaped enlargement, or "ampulla of Thoma." The venous sinuses, similarly, open directly into the pulp interstices. The pulp channel, interposed between an ampulla of Thoma and a venous sinus, may assume the appearance of an intactly walled arteriovenous connection when the spleen is contracted. Thus, the structurally "open" circulation of a relaxed or distended spleen is converted into a functionally "closed" vascular

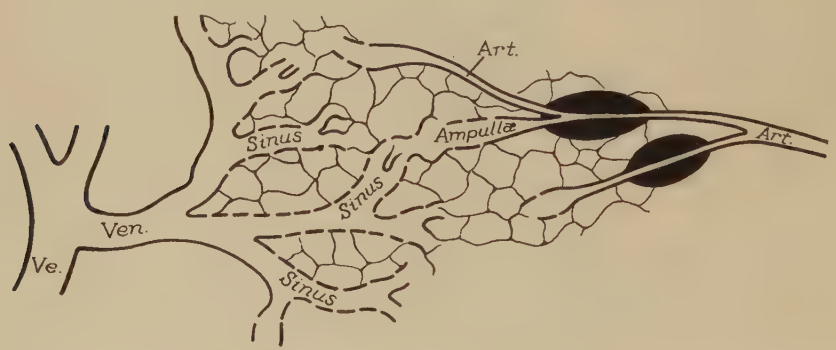

Fig. 2 Diagram of splenic circulation modified from figure 54 of Hartmann ('30).

circuit by contraction of the capsule and trabeculae. The factors fundamentally controlling circulatory changes in the spleen are trabecular and capsular contraction, together with arterial constriction. They found no powerful sphincter action anywhere in the red pulp, as Knisely had described.

Although MacKenzie, Whipple and Wintersteiner published a diagram of their conception of the splenic circulation in the cat, our figure 2, modified from Hartmann ('30) after Neubert ('22), fits more closely the descriptions and drawings of their observations on the mouse spleen.

Before publication, MacKenzie informed Knisely that he and his colleagues had been unable to confirm Knisely's

observations. Knisely thereupon visited MacKenzie's laboratory, and using MacKenzie's experimental set-up, was unable to demonstrate the cyclical storage activities of the venous sinuses. The splenic circulation observed at that time by both investigators was rapid and constant.

In the meantime, beginning in 1939, we had been continuing and extending studies on the splenic circulation begun when one of us (N.L.H.) had been a colleague of Knisely at the University of Chicago. We had revised the technic of exteriorizing and studying the spleen in the course of our attempts to improve the transillumination method, and had regularly confirmed many of Knisely's published observations, particularly the storage cycles of the venous sinuses.

In addition, Knisely and Hoerr had demonstrated the long slender "shunt" capillaries in spleens prepared by the frozen-dehydration method, an observation which had not been hitherto published. Furthermore, after attempting to eliminate all sources of small vibrations from our experimental set-up, we had had no difficulty in following the refractile double lines lining the blood stream from artery to vein, described by Knisely as characteristic of intactly lined blood vessels in any transilluminated tissue. We could not understand how MacKenzie saw blood cells pour out of arterial capillaries, to "trickle onward" through "swamp-like pulp zones," or why he described blood flowing through pulp spaces, which were "tortuous, utterly irregular and inconstant channels." In our preparations, the vascular pattern of a certain microscopic field either remained constant or reverted repeatedly to an identical form for as long as 10 hours.

In view, therefore, of the complete disagreement between the two reports of competent and experienced investigators, we decided to restudy the problem, repeating as closely as we could the experimental conditions of both reports, to see whether we could determine what differences in technic, if any, might have led to the discrepancies between the results.

METHODS

During the course of this study, extending over the past 10 years, we have studied the splenic circulation in each mouse for at least an hour, occasionally for periods as long as 8 to 10 hours, in over 350 white mice of different strains and ages, weighing from 8 to 25 gm, the majority of them weighing 18 to 22 gm. They were studied at various intervals after feeding, and after periods of fasting up to 24 hours. The mouse colony was maintained, in the main, on Purina dog chow and water exclusively. In most instances, the mice were examined after the experiments for gross evidences of disease; every attempt was made to use only healthy mice.

In preparing the mouse spleen for observation by transillumination, the following procedure was used most frequently, although many variations of the operative approach, anesthesia, and illuminating technic were tried. The mouse was anesthetized about 30 minutes to 1 hour before operation to insure, if possible, a stabilization of physiological processes. The barbiturates are known to depress the blood pressure temporarily, and in an animal as small as a mouse, respiration and heart rate may be variable for about a half hour after the anesthetic is given.

Several of the barbiturates, including sodium amytal, barbital, sodium pentobarbital, and Dial were used. Of these Dial was found to be the most satisfactory because the period of anesthesia was longer, and the respiratory movements were quieter and more regular. One injection produced anesthesia lasting 3 to 4 hours, before a second injection was required.

After anesthesia is complete, the fur over the splenic region is clipped closely with scissors. In some instances the skin was shaved, or cleared of fur with a depilatory. An incision through the skin only is then made just below and parallel to the last rib on the left side, extending from a point about 5 mm lateral to the spinal column to a point on the midclavicular line.

The mouse is then mounted under the spleen tray illustrated in figure 3. This tray is a rectangular frame of lucite (A) with a thin sheet of celluloid (B) cemented to one side to form the bottom of the tray. The sides of the tray are low enough to allow clearance of the microscope objectives and

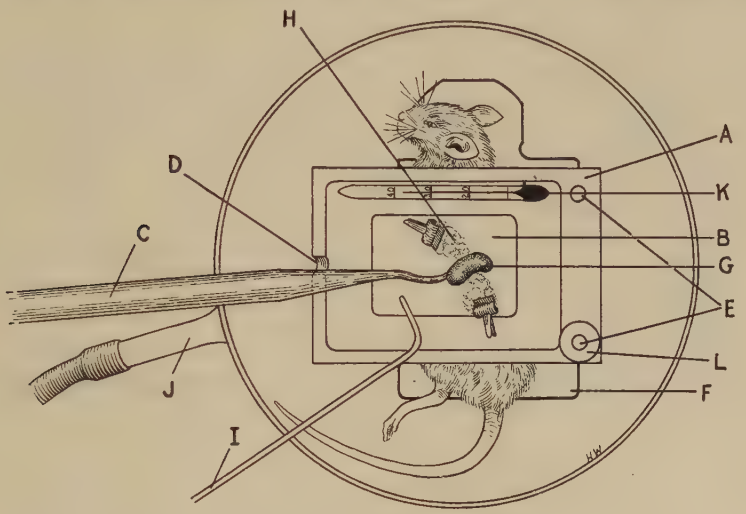

Fig. 3 Details of tray for suspension of spleen.

(A) Lucite spleen tray.
(B) Thin celluloid bottom of tray.
(C) Solid quartz rod.
(D) Notch cut in end of chamber for exit of Ringer's solution.
(E) Supporting posts for spleen chamber.
(F) Non-conducting base (bakelite).
(H) Cotton pellet clamped with card slip.
(I) Cannula for delivery of Ringer's solution.
(J) Drain pan with outlet tube.
(K) Thermometer, calibrated for total immersion.
(L) Thumb nut for adjusting height of chamber.

to permit the quartz rod (C) to be inserted at an angle not too far from the horizontal, but high enough to hold sufficient fluid to cover the exteriorized spleen completely. The capacity of the trays we have used is 20 to 30 cm^3.

A notch (D) is cut in one end to hold a wick for drainage of the solution surrounding the spleen. The frame is sup-

ported by two posts (E) at one end, on which the tray can be raised and lowered easily. The tray is held firmly at any desired height above the base by a spring and thumb nut on one of the posts. The posts are fastened to a bakelite base (F). A circular hole 9 mm in diameter is cut in the center of the celluloid bottom of the tray, the edges being carefully smoothed.

The skin around the incision is cemented with rubber cement to the bottom of the tray, leaving only the deep tissues of the incision exposed through the hole in the tray. The incision is then extended through the muscles and peritoneum. Bleeding is controlled by cautery. It is important to have no bleeding, since the loss of even a minim of blood is sufficient to alter appreciably the splenic circulation of a 20 gm mouse.

Ringer's solution at 37.5°C. or 38°C. is then run into the tray. The tray is then gently squeezed against the mouse, and the lower abdominal wall and back are gently pressed with cotton covered applicators. In most cases the spleen pops out of the abdomen and into the tray with little difficulty and without being touched. Usually the anterior margin of the lower pole of the spleen presents first, and a gentle prodding of the animal's back and belly exposes the posterior margin and then the entire spleen. The spleen (G, fig. 3) in being thus exteriorized is always surrounded by warm saline solution, and is not subjected to local trauma of manipulation. In most instances the gastrolienal ligament is long enough to permit good exposure of the spleen without tension on the vessels of the pedicle.

Further immobilization of the spleen is secured by gently pushing absorbent cotton pellets, weighted with card clips, or held down by rubber bands cemented to the margins of the hole in the tray (H, fig. 3), under the margins of the spleen until the movements caused by respiration are largely eliminated. Careful adjustment of the height of the tray above the mouse is also helpful in reducing the movements produced by the excursions of the diaphragm. Pressure by

the tray on the body of the mouse must be avoided. In some mice the gastrolienal ligament is shorter, and it may be partly incised without hemorrhage and without, as far as we can determine, altering the splenic circulation.

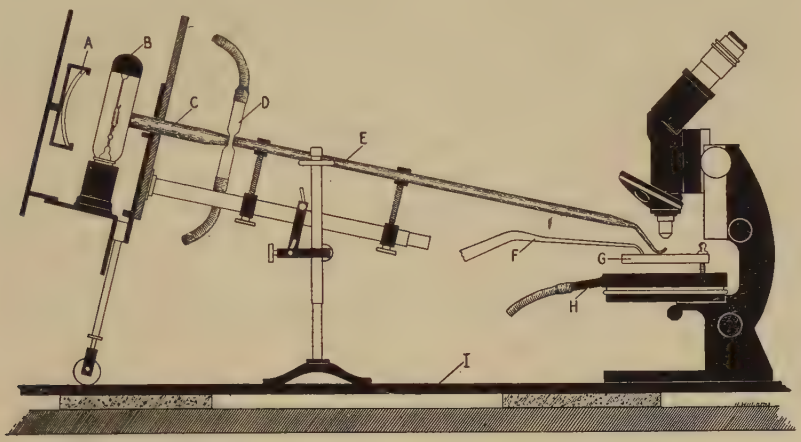

Fig. 4 An example of transillumination apparatus.

(A) Concave mirror adjusted so its point of focus is approximately at bulb end of rod C.

(B) Four hundred to 1000 watt, Mazda T-10 or T-20 projection bulb.

(C) Four inch by one-half inch quartz rod drawn to five-sixteenth inches at lower end.

(D) Light filter tube. See text.

(E) Long solid quartz rod drawn to desired size and shape at lower end.

(F) Tube delivering Ringer's solution from constant temperature bath to spleen tray.

(G) Spleen tray. For details see figure 3.

(H) Drain pan fastened to circular stage of microscope.

(I) Boiler plate or armor plate three feet square and one-fourth inch thick supported on sponge rubber pads.

The tray (see G, fig. 4) is placed on the stage of the microscope, in a larger tin drainage tray (H, fig. 4, or J, fig. 3) fastened to the circular stage, so that it can be moved by the centering screws of the stage. The tip of a glass cannula (F) is inserted in one corner of the tray (see also I, fig. 3); this cannula is connected to a supply of Ringer's solution, maintained at pH 7.4, and warmed in a constant tempera-

ture bath so that the temperature of the solution surrounding the spleen can be maintained at any desired level. The rate of flow is then adjusted at from 5 to 20 cm^3 per minute. The larger fluid flow insures that any heat formed in the spleen by the absorption of light from the quartz rod is immediately conducted away from the tissue. However, a rapid flow of immersion fluid sometimes causes enough eddy currents in the tray to cause undesirable vibrations in the spleen, so a slower exchange of Ringer's solution is sometimes necessitated.

The temperature of the Ringer's solution surrounding the spleen is measured by an accurately calibrated two inch total immersion thermometer (K, in fig. 3). Once the temperature of the constant temperature bath, and the rate of flow of the Ringer's solution are established, the temperature of the fluid surrounding the spleen can be precisely maintained within a range of less than 0.3°C. variation. The temperature was checked by placing the thermometer against the spleen as well as in other parts of the tray. There is enough flow of solution to keep the temperature uniform throughout the tray. The solution is drained, by the wick mentioned above, from the spleen tray to the drainage tray, without splashing on the mouse. Thus the mouse is kept dry and warm at 72°–78°F. regardless of the temperature at which its spleen is maintained. Of course, there is some leakage of Ringer's solution from the spleen tray to the peritoneal cavity; any considerable alteration from 38°C. of the temperature of the Ringer's solution wash will have an effect on the intra-abdominal temperature of the mouse.

In the early experiments, we used the hollow-tipped quartz rod (Knisely, '37, '38) which delivers Ringer's solution directly to the illuminated area. This, however, did not furnish enough light for photographic purposes, so for some years we used a solid rod. With the set-up described above, the spleen floats freely enough, and there is a rapid enough exchange of solution, so that there is always a sufficiently thick film of constantly renewed fluid between the tip of the rod

and the under surface of the spleen. The earlier hollow-tipped rod had the further disadvantage of causing slight vibrations in the spleen; the lumen of the tip was so small that the stream of Ringer's solution beat against the under surface of the spleen unless the rate of flow was quite slow. Recently, we have been using the hollow-tipped rod pictured in figure 5. This rod differs from the one originally devised by Knisely in that the solid rod and the tubing are not fused completely; the rod and tubing, with accurately plane facing ends, are brought into contact and slightly stuck together with a very thin film of surface fusion. Practically no light

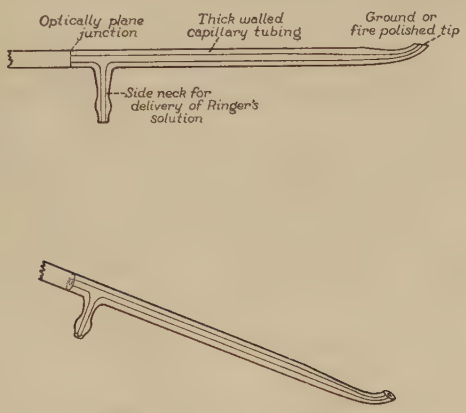
Optically plane junction
Thick walled capillary tubing
Ground or fire polished tip
Side neck for delivery of Ringer's solution

Fig. 5 Improved hollow-tipped fused quartz transillumination rod. See text.

is lost at this junction, and since the tubing is thick-walled capillary tubing, more light is transmitted down the tubing to the tip. For most experiments, the tip is left purposely larger than was our earlier custom. This new design of fused quartz rod delivers almost as much light for photography at high magnifications as the solid rod. Further, the lumen of the tip being larger, the stream of Ringer's solution does not beat against the illuminated tissue with so much force as the old design.

The light source for the early experiments was a 400 watt Mazda T-10 projection bulb, cooled by forced draft. All types

of air-cooling of the light introduced minute vibrations which were transmitted to the spleen; this problem of vibration will be discussed later. Therefore, we gave up the forced draft cooling of the bulb, and built an open lamp-housing. Since we usually run the lamp at only half or two-thirds the rated voltage, controlled by a variable rheostat, and use the full voltage for only a few seconds at a time, we have been able to use even 1000 watt T-20 bulbs without forced draft cooling. A concave mirror (A, fig. 4) so placed that its focal point is in the end of the quartz rod, approximately doubles the light input of the rod.

Another way in which light input can be increased is to use a large diameter (one-half inch) segment of rod at the bulb (C, fig. 4). This can be fused to the smaller rod, or left as a separate segment, about 4 inches long, to permit the insertion of a filter (D) between the two rods. The rod (E) which extends to the spleen is one-fourth or five-sixteenths inch in diameter and 10 to 12 inches long. The rod was held in place by wooden clothes pins (J) mounted on a length of coiled spring. This flexible mounting of the rod lessens the danger of breakage, but allows vibration of the rod, so that now we use a rigid mounting.

The filter (D) consists of a glass tube in which a segment has been flattened so that the light passes through two parallel plane walls. A solution of any desired color, usually 1% aqueous solution of light green, is circulated continuously through the tube. This prevents the filter from heating. Since Knisely ('37) found that the T-10 and T-20 projection bulbs transmit a negligible amount of ultraviolet light, and that variation of wave-length causes no detectable effect on capillary circulation, we, in recent years, no longer transmit filtered light to the tissue, but merely insert gelatin filters of the desired color into the ocular of the microscope.

The microscope, quartz rod holder, and lamp housing are all mounted on a sheet of steel armor plate (I, fig. 4), three feet square and three-eighths of an inch thick, weighing about 150 pounds. This sheet is separated from the table by sponge

rubber pads, or a combination of cork and sponge rubber. The table is a large, solid one, weighing 400 pounds, and in turn is separated from the floor by large and thick soft rubber stoppers. The constant temperature water bath, with its stirring rod which produces vibrations, is separately supported on a heavy steel rack mounted on rubber-tired casters, entirely independent of the microscope table. The motion picture camera, also independently supported from the floor, never touches the microscope, so that vibrations from its motor drive can be transmitted to the microscope only through the floor. The floor is concrete, 10 inches thick, and the room is 300 feet from the nearest street. We have tried in every way to prevent external vibrations from reaching the mouse. Even so, we have sometimes detected the vibrational effect of a person walking along the corridor outside the room, when we were working at 600 to 800 × magnifications, and accordingly have conducted a majority of our experiments at night. A good test for the presence of minute vibrations is to place a dish of mercury on the spleen chamber, and look for ripples on the surface, under all the varying conditions of the experiment.

In order to get the clearest possible perception of the microscopic image, it is important to prevent any extraneous light from reaching the eye of the observer. To this end, we shaded the lamp housing, covered the quartz rod with black paper or cloth to shut off the light lost along the rod, and worked in a completely darkened room or at night.

OBSERVATIONS

The red pulp in the mouse spleen consists of relatively narrow anastomosing regions surrounding the white pulp. Under low magnification, it may have the appearance of a mass of red blood cells, some areas being densely red, or in a contracted spleen, it may consist of alternate patches of pale red and almost white. There is no sharp boundary between white and red pulp.

There are so many vessels in the red pulp, that when the circulation is active, the tissue seems under low magnification to be composed almost entirely of blood vessels, with only narrow partitions of extravascular tissue (see fig. 6). Scattered throughout the red pulp there are small elliptical regions, about 15 to 30 μ by 40 to 80 μ, which are quite red, and bounded by a definite line. They often have the shape of short cucumbers or sweet potatoes; as can be seen under higher magnification, these are the venous sinuses.

The central arteries of the white pulp, on reaching the red pulp, usually divide into 2 to 6 branches, the red pulp or

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Figs. 6 to 20 inclusive are reductions of sketches of splenic circulation drawn from the living spleen. An ocular micrometer, or a squared eye-piece micrometer, or the two in combination, were used in making the drawings. The original sketches were made on graph paper, or cross-ruled paper, following the measurements made with the ocular micrometers. Although the drawings were made free-hand, they are fairly accurate, and could be made more rapidly and without the difficulty which would be entailed in attempting to use a camera lucida.

art., artery or arteriole.
P.A., artery of the red pulp.
cap., capillary. In some instances these may be sinuses in a conducting phase. See "sin."
cap. sh., capillary shunt.
sin., sinus. Only designated as such if storage or filtration was observed.
a.v., afferent vessel of a sinus.
E.C., endothelial cell nucleus.
e.v., efferent vessel of a sinus.
ven., venule.
ve., vein.
. . . . Vessel deeper in tissue than the other vessels being drawn. Does *not* indicate stomata in vessel wall.
———— Completely intact vessel walls. No vessels were drawn unless the walls could be seen.
———) Indicates that a vessel entered the deep tissue at this point and could not be followed further.
—X—X— Points at which sphincter action was observed.
——→ Arrows indicate the direction of blood flow.
←——→ Double arrows indicate that a reversal of blood flow occurred during observation.
White areas represent the approximate region of the red pulp tissue, stippled areas the lymphatic or white pulp tissue.

penicillar arteries, which extend into the red pulp for 10 to 15 μ, and then themselves divide into 2 to 5 branches, which are capillaries. (See figs. 7, 8, and 9.) If more than two capillaries arise from a red pulp artery, the termination

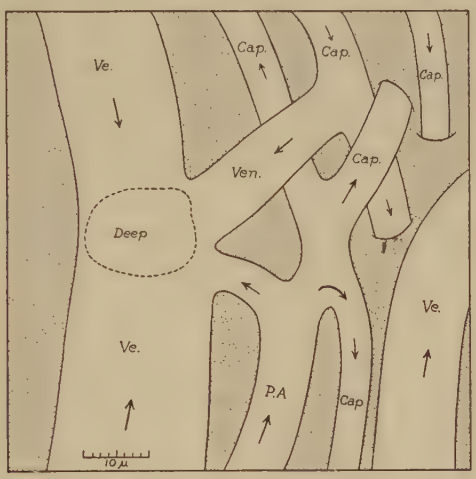

Fig. 6 Small area in red pulp 72 μ on each side. This shows the number of vessels which may be present in one plane of focus in such an area. By changing the focus of the microscope we could see more vessels.

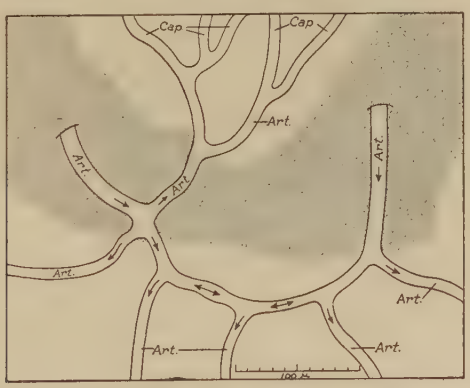

Fig. 7 Different types of central artery branchings.

of the artery may be ampulla-shaped. (See fig. 10.) There is, however, no discontinuity of the refractile lines bounding the blood stream; these smooth lines can be followed from the arteries along the capillaries.

We do not know exactly what these refractile lines represent in the fixed and stained tissue. They certainly represent some kind of endothelium, because in some instances bulges into the lumen can be seen, which we interpret as endothelial nuclei. These "bulges" are grey elliptical masses, about 3

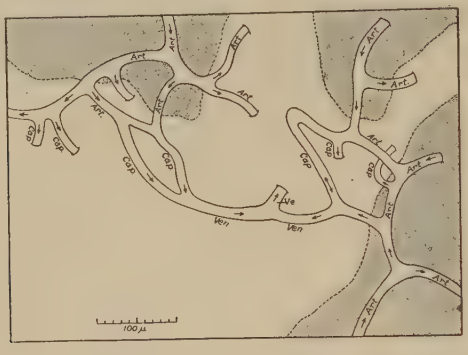

Fig. 8 Different types of central artery branchings.

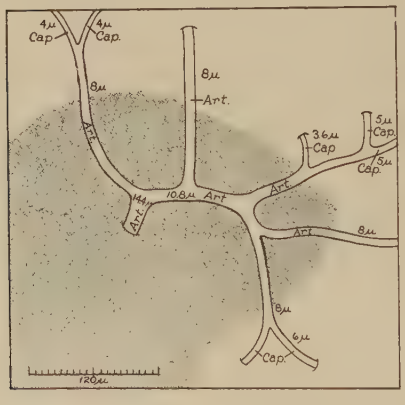

Fig. 9 Different types of central artery branchings.

by 15 μ in size which project into the lumen, so that when the rate of blood flow is slow, individual blood cells can be seen to bounce against them. The refractile lines of the vessel walls may also represent reticular fibers, in the case of capillaries, or the entire intima, in the case of arteries.

The capillaries, which extend in all directions from their origin at the ampulla-like terminations of the penicillar arteries, vary greatly in length. Some may be only 20 μ long,

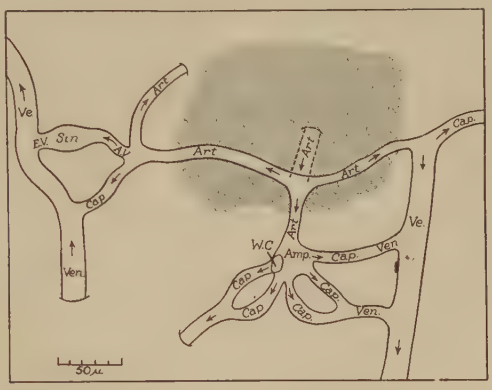

Fig. 10 An ampulla of a red pulp artery. The white cell was in the indicated position for 30 minutes.

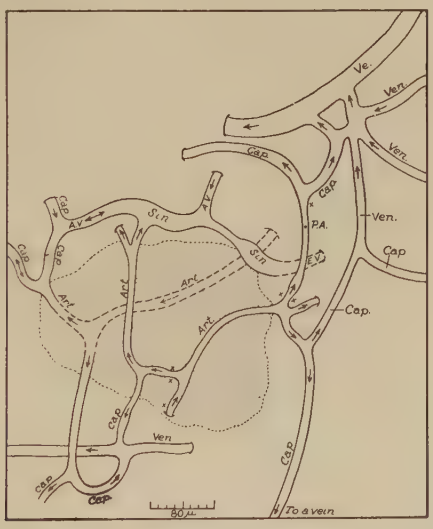

Fig. 11 Different modes of termination of capillaries.

others may be followed for 100 μ. They terminate either in venous sinuses, or in venules, or may themselves combine with another capillary to form a venule. (See fig. 11.) The

diameters of the capillaries are usually greater than those of the penicillar arteries, varying from 5 to 10 μ in a relaxed spleen. When the spleen is contracted the diameters of the capillaries tend to decrease, and some disappear. That is to say, when their circulation stops, and they are emptied of red cells and plasma, they cannot be seen in the living spleen.

The course taken by a capillary may be straight, curved, or very tortuous. If they are tortuous, it may be impossible to trace them to their terminations, partly because they may run under other vessels, partly because they may extend beyond the limit of focusing. Capillaries may turn directly away from the microscope at their origins, which may give

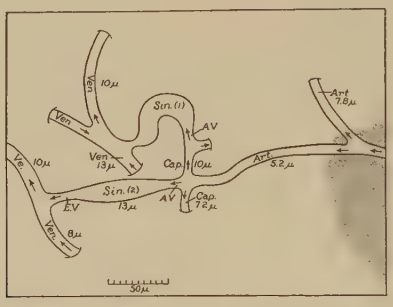

Fig. 12 Simple type of venous sinus.

the ampullary termination of a penicillar artery either the appearance of ending in a sac, or of pouring its blood into the pulp.

Some of the capillaries which run parallel to the plane of focus of the microscope can be observed to terminate in venous sinuses. (See fig. 12.) The walls of the sinus in the contracted spleen are difficult to see, as they may be thin, dark lines. When the sinus is dilated, the wall becomes thinner and more refractile, and is more easily seen. In either case, however, the smooth, bounding line is continuous with the refractile wall of the capillary.

Sinuses may be single (see figs. 12 and 13), with one afferent capillary and one efferent venule, or they may be in

multiple systems, as Knisely described them. Not all of them, of course, lie in a plane parallel to the plane of focus of the microscope, so that it is possible to see the entire sinus clearly only in favorable cases. They may also be irregular in shape, due to connections with vessels on the sides, instead of only at the ends. (See figs. 14 to 17.)

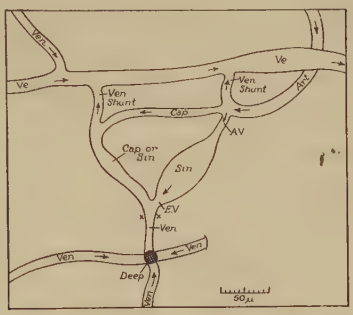

Fig. 13 Simple type of venous sinus.

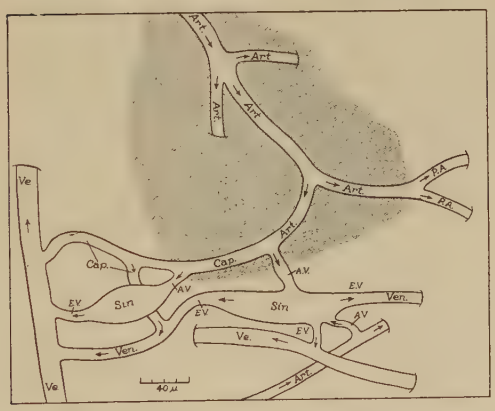

Fig. 14 Complex venous sinuses.

Contractions of the spleen have been noticed in nearly every mouse studied. In the mouse these contractions are usually so slight as to be undetectable grossly, but may be noted under the microscope by the changing relations of the blood vessels. The contractions are irregular, not rhythmic. They do not materially affect the splenic circulation, unless

they are extreme, as with the splenic contraction caused by adrenalin. In this extreme contraction, many of the venous vessels are emptied, and the spleen becomes pale.

The blood flow through the red pulp or penicillar arteries may be quite rapid, but it is extremely variable, and often stops completely. At times, widely scattered or isolated blood cells pass along, indicating that the flow is mainly plasma. The diameter of these arteries is small, being less than the

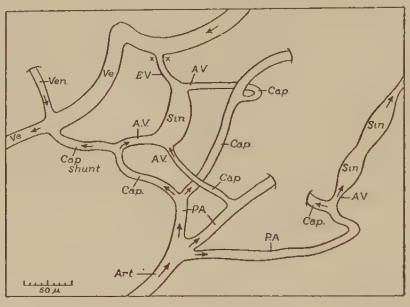

Fig. 15 Complex venous sinuses.

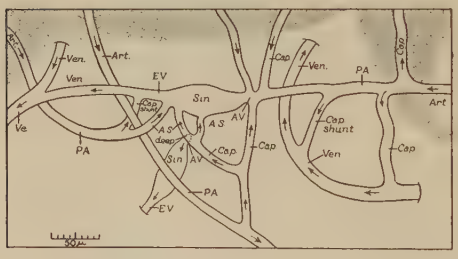

Fig. 16 Complex venous sinuses.

diameter of a red blood cell most of the time, so that the cells are greatly distorted in passing through. White cells often plug the proximal end of an artery for a few seconds.

The penicillar arteries have the most powerful sphincter action seen in the splenic arterial tree. Sometimes the entire length of an artery will contract strongly, while neighboring arteries continue to have a rapid flow. Constriction, even to complete obliteration of the lumen, at either the afferent

and efferent ends of the penicillar arteries has been seen, a solid column of blood cells remaining proximal to the constriction, or an occasional red cell "squeezing" through the constriction. These arteries can often be seen in their entirety.

As the blood passes from the narrow penicillar arteries to the wider capillaries the rate of flow is sharply reduced. The individual red cells can usually be seen as they pass along the vessel, but occasionally the rate of flow may rise greatly.

Some of the long, more or less straight capillaries which can be seen to terminate in venules have a fairly constant

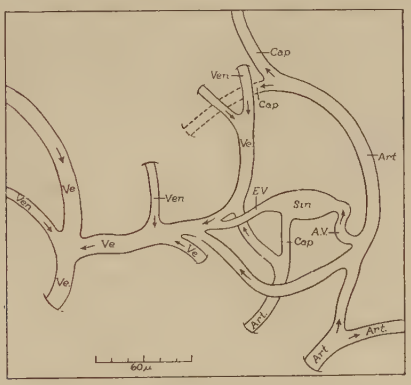

Fig. 17 Complex venous sinuses.

blood flow, as seen in several hours of steady observation. These would appear to be shunts or by-passes around sinus systems which go through prolonged storage phases. (See fig. 18.)

Because of the tortuosity of the capillaries, it often seems at first glance that the red cells are migrating through the extravascular tissue. Frequently, series of red cells seem to pop out of vessels in the direction of the microscope and then wander aimlessly across the field. (See fig. 19.) Careful examination under higher magnification, however, will reveal the cells to be in capillaries, which twist and turn more or less in a plane parallel to the plane of focus. The extreme

thinness of the capillary walls, and their lack of contrast with extravascular tissue, often make them difficult to define. Under these circumstances, increasing the obliquity of the light from the quartz rod is helpful.

All of the phases of activity of the sinuses described by Knisely have been observed. The efferent end of a sinus

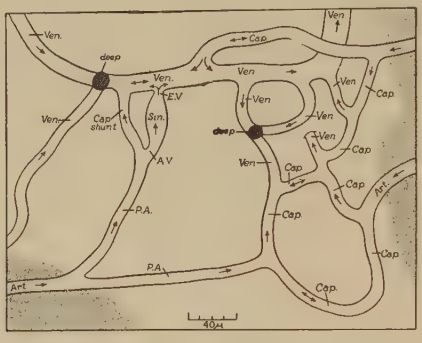

Fig. 18 A capillary shunt.

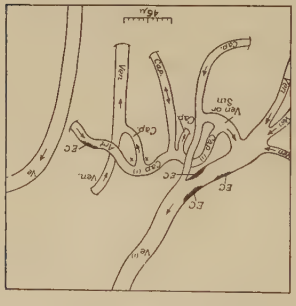

Fig. 19 A complicated capillary pattern.

may constrict, so that no blood passes into the efferent venule. Blood continues to flow into the sinus, which increases in diameter. At the efferent end of the sinus, the cells are packed more closely together, until they form a dense, red, homogeneous mass, and the individual cell outlines may no longer be seen. When a sinus contains only packed red cells, the flow may stop for varying lengths of time from a few

minutes up to several hours. White cells can be seen easily in the densely packed red mass.

The emptying of a sinus may occur suddenly and completely, slowly, or by spurts. The stored cells enter the venule in small densely packed clumps, which suddenly break apart to individual cells. The diameter of the sinus again decreases to about 10 μ, and conducts blood rapidly, so that it is impossible at times to distinguish it from a capillary, until it fills and dilates again.

The continuous filtering phase of the sinuses described by Knisely has also been observed. In this phase, the sinus

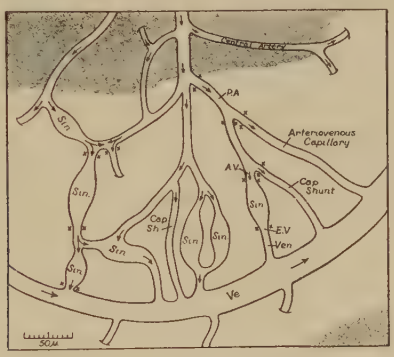

Fig. 20 Diagram, summarizing the main types of arteriovenous connections which have been observed in the red pulp.

conducts blood continuously, but as the blood cells pass through the sinus, they come closer together. This approximation of the cells appears to be due to loss of plasma, since there may be no change in the diameter of the sinus, nor any evidence of a peripheral plasma layer. During this phase of activity, the sinus may for long periods have approximately twice the diameter of a capillary (15 to 20 μ).

The storage phase of the sinus usually has the longest duration, the filling and emptying phases progressing rapidly as a rule. The conducting phase and the continuous filtration activities are predominant in the contracted spleen; the storage phase is more frequent in the dilated spleen. The

most frequently seen of the various types of vascular patterns and activities we have observed here have been summarized by the diagram of figure 20.

DISCUSSION

Snook ('44) stained guinea pig spleens for reticular fibers in order to define the venous sinuses, and then made a wax plate reconstruction of a portion of a spleen. No one, on looking at the photographs of his sections, can doubt that Snook has demonstrated the "essential structure" which nearly every investigator has agreed is characteristic of a splenic venous sinus. These venous sinuses, when reconstructed, as Snook says, and as his photographs clearly show, "are in fact shaped like sweet potatoes or cucumbers" precisely as Knisely had described them, tapering from 30 to 50 μ in diameter at the widest part to 10 to 20 μ at the point where they join another sinus or vein. Snook also confirmed the "multiple sinus" structures described by Knisely.

It becomes apparent immediately then, from studying the descriptions and drawings of MacKenzie and his coworkers, that what they described as "pulp channels" are in fact the venous sinuses, and that what they termed venous sinuses are, in actuality, only the efferent ends of the venous sinuses plus the first parts of the venules, or even the venules alone.

If this difference in terminology is recognized, then one is led to the conviction that although MacKenzie, Whipple and Wintersteiner state that no blood storage or other cyclical function can be attributed to the venous sinuses, they have in truth recognized the storage phase of activity. On page 424 of their paper, they state,

> "It has been our experience that the several components of venous sinus cycles, as described by Knisely — namely, filtration-filling, storage, emptying and conduction phases — may be studied in any relaxed spleen as they take place, not in the venous sinuses but in the intercellular channels of the pulp. The periodicity of the component phases, except in its relationship to rhythmical splenic contraction, is totally irregular. We do not know how long some pulp

spaces may retain red cells. Upon occasion, some have been seen to do so for several hours. Most pulp channel cycles are extremely brief. The same space may accomplish 3 or 4 separate storage phases within 10 minutes.''

It may be mentioned again parenthetically that storage phases predominate in expanded spleens. But several contradictions may puzzle the reader of this statement of MacKenzie and his coworkers. They insist throughout their paper that blood is emptied from the spleen largely by capsular and trabecular contraction. How then could a pulp channel undergo a storage phase for several hours, presuming that rhythmical splenic contraction continued at the rate of one per minute in the cat? And if the periodicity of the various phases is dependent upon splenic contraction, which is rhythmical, how could it be ''totally irregular?''

When one has become accustomed to examining the living spleen under high magnifications, an educational process which is by no means simple, it is difficult to understand why MacKenzie, Whipple and Wintersteiner saw the ''pulp channels'' or as we have come to believe, the sinuses, as ''tortuous, utterly irregular and inconstant channels, lined by fixed and wandering cells and by reticulum.'' Why did they not see these channels as we have observed them, as smoothly bounded channels, which keep the same pattern through successive dilations and constrictions, hour after hour?

One possible explanation of their observation that the channels were lined by fixed and wandering cells may be that their animals had suffered too much trauma. They made a larger incision than we did, the stomach was drawn downward and outward into the incision, peritoneal bands connecting the spleen with the diaphragm, and the gastrolienal ligament were divided. From their figure 1, it is apparent to us that they mobilized the spleen much farther from the animal's abdomen than we did, and that possibly it had been somewhat turned on its pedicle. It may also be mentioned at this point, as we shall endeavor to show in a subsequent paper, that MacKenzie maintained the spleen at a tempera-

ture perhaps as much as 2°C. higher than the normal intra-abdominal temperature. One of the first results of trauma, as we have seen often, is that the leucocytes, instead of rolling along easily in the vessels, stick to the vessel walls, and are then buffeted by the red cells. This, to be sure, presents an appearance of an irregular channel.

MacKenzie and his coworkers nowhere say what precautions they took against minute vibrations. They do state that the lamp was prevented from overheating by a current of compressed air. We found that any method of forced draft ventilation of the lamp produced vibrations in the lamp housing which were communicated to the quartz rod and then to the spleen. The vibrations were sometimes so minute as to be at the limit of microscopic resolution, less than 0.5 μ, but also up to a micron or slightly more. These vibrations can be fatal to good definition, as we proved over and over again on many fields under 600 × to 800 × magnification, turning the air on and off a number of times without removing the eyes from the microscopic field. A vessel wall which was difficult to see to begin with, would instantly disappear when the fan was turned on.

The reader may wonder at this point why we belabor so obvious and possibly minor a problem as the absolute prevention of vibration. If we can show the great importance of the fact that absence of *small* vibrations is more important than any other factor in the transillumination studies of living tissue under high magnifications, this paper will have served its purpose. Perhaps an analogy or two will help. A fisherman may stand up and rock the boat, or row through rolling waves, and still be able to see the rocks on the bottom of an 8-foot pond. But let a light drizzle of rain pepper the surface of the water, and the fisherman can no longer see anything below the surface. Or picture a man in a barber chair, reading a newspaper. The barber may, in shampooing his hair, shake the man's head, but the latter will still be able to read. Let the barber, however, apply an electric vibrator to his scalp, and immediately the newsprint becomes blurred to him.

Unless these annoying infinitesimal vibrations are prevented, a penicillar artery, ending in branches which abruptly turn out of the plane of focus, might well appear to open into pulp spaces. We have mentioned above that the original Knisely hollow-tipped rod, delivering 4 to 6 cm^3 per minute of Ringer's solution through a very small jet, would often buffet the tissue. This alone might explain such statements as (MacKenzie, p. 412) "The arterial capillary ampullae communicate directly with the pulp interstices, by way of numerous apertures, which we are unqualified to describe, except to say that their size is adequate for the unobstructed transmission of whole blood." In the light of our long acquaintance with the transillumination technic, 6 years for one of us (H.M.P.) and 15 years for the other (N.L.H.), we would say that such statements arise through lack of adequate microscopic definition. And of all the causes for this lack, minute, practically undetectable vibration is the greatest. The larger vibrations, such as those produced by respiratory movements do not so seriously hinder clear vision, because the observer can learn to become accustomed to them.

MacKenzie states that the arterial capillaries reveal no sphincter mechanisms, and that the efferent ends of the venous sinuses remain patent under all normal conditions. We hope in a subsequent paper to show that MacKenzie was dealing with spleens maintained at temperatures higher than normal, and in overheated spleens the circulation tends to become vigorous, rapid, and constant.

However, as regards the filtering function of venous sinuses, in the following quotation from MacKenzie's paper, page 424, change only the one term "pulp interstices" to "venous sinuses," and the statements would be completely agreed to by Knisely and ourselves.

"Generally speaking a variable amount of hemoconcentration develops as blood traverses the *pulp interstices.* (Italics ours.) Although certain of the largest and most direct channels permit red and white cells to pass through them practically unobstructed, the majority of the pores, because of their size, contour and course, more or less

diminish the rate of flow. The *meshes of the pulp* (italics ours) thus effect a form of filtration, tending always to separate red cells from plasma. Most densely packed are the stationary erythrocytes. However, in innumerable passages where red cells are not completely arrested, it may be seen that, as the blood columns approach venous tributaries, individual erythrocytes lie closer to one another in the fluid that carries them along. In a relaxed spleen, the filtration effect of the pulp is obvious. In a contracted spleen, it appears to be more or less eliminated."

When we have been able to see capillaries appear and disappear in great numbers in a given field of the red pulp, that is, for blood flow in them to start and stop, but keep exactly the same pattern hour after hour, up to 10 hours of observation — we have been forced to the conclusion that they are not haphazard channels in the pulp, but definite, morphologically intact and integrated components of the vascular system.

CONCLUSIONS

1. The intermediary splenic circulation of the red pulp of the white mouse spleen is essentially as Knisely described it, both structurally and functionally. The blood passes through lined, intact, constant blood vessels which join the arterial tree with the venous system, and have the peculiarity of leaking blood plasma, particulate matter, and even some blood cells, to the extra-vascular spaces of the red pulp. This special type of circulation might be designated as "separatory circulation."

2. The circulation in the mouse spleen is relatively independent of splenic contractions. The storage phases of venous sinuses are more common in expanded spleens, the continuous filtering and conducting phases in the contracted spleens. The continuity of the intermediary vessels is not disturbed by the state of expansion or contraction of the spleen.

3. Minute vibrations are the greatest hindrance to good microscopic definition at high magnification. These vibrations are not visible grossly. The vibrations make resolution of the

endothelial lining of vessels difficult or impossible. These vibrations are transmitted to the preparation through the building and supporting table and must be dampened by suitable insulating materials.

4. The exacting technic of the transillumination technic is discussed.

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THE EFFECT OF ENVIRONMENTAL TEMPERATURE CHANGES ON THE CIRCULATION OF THE MOUSE SPLEEN [1]

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In a previous paper (Peck and Hoerr, '51) we described the intermediary circulation in the red pulp of the mouse spleen. Our observations confirmed in most essentials those which Knisely published in his first paper on the spleen (Knisely, '36). The splenic circulation is mediated by lined, intact, constant blood vessels which join the arterial tree with the venous system, and have the peculiarity of leaking blood plasma, particulate matter, and even some blood cells to the extravascular spaces of the red pulp. We suggested that "separatory" circulation might be a short designation of this special circulation.

In the paper mentioned, some of the discrepancies between the reports of Knisely, '36, and of MacKenzie, Whipple and Wintersteiner, '41, were discussed. Possible explanations of some of the opposing conclusions in these two papers were offered. In this paper, we hope to explain some of these differences more fully in describing the effect of temperature changes on the exteriorized spleen, and in discussing methods of temperature control in the study of the spleen by the Knisely fused quartz rod transillumination technic.

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Knisely, '36, described cyclic activities of the splenic venous sinuses. A sinus may pack blood cells closely together by leaking plasma through its wall, and after it is filled and expanded with packed cells, may shut off both its inflow and outflow for long periods, storing cells which are thus sequestered from the circulating blood stream. After a longer or shorter storage phase, a period dependent upon unknown factors, a sinus may then dilate its constricted efferent end, and empty the cells slowly or rapidly into the venules. It may then conduct blood steadily for a variable period, before again packing and storing cells. Knisely divided these cyclic activities into what he called filtering-filling, storage, emptying and conducting phases.

Knisely also described a continuous filtering activity of the venous sinuses, in which the sinus, although continuing to conduct blood, leaks plasma to the extravascular spaces, so that the blood cells are brought more closely together as they traverse the sinus. Knisely further discussed the mechanisms whereby these venous sinus activities were effected, describing sphincter actions in the arteries, arterial capillaries, and at both the afferent and efferent ends of the venous sinuses.

MacKenzie, Whipple and Wintersteiner ('41) observed the circulation of the living spleen by the same transillumination method, and mainly in the mouse, as had Knisely, but came to quite different conclusions. They stated that the intermediary splenic circulation is "open," that the only connections between arteries and veins are the inconstant interstices of the red pulp network. They denied further the sphincter activities reported by Knisely, and the cyclic activities of the venous sinuses. They concluded that the red blood cells enter the venous system from the pulp spaces through contraction of the capsule and trabeculae.

We had begun in 1939 to restudy the question of the splenic circulation in order to lay the foundation for some studies on phagocytosis in the spleen, and had without undue difficulty seen most of the structures and activities described in Knisely's first paper on the spleen. When, therefore, in 1941, the

completely contradictory report of MacKenzie, Whipple and Wintersteiner appeared, we re-evaluated the methods we had developed, and then repeated as closely as we could the methods detailed by both previous papers.

It should be put on record, we believe, that when the investigator passes from a study of circulation in a thin organ or tissue, such as mesentery or omentum, tongue or web of the foot in the frog, rabbit's ear, bat's wing, or tadpole's tail, to the transillumination of a thicker, more opaque organ, such as spleen, liver, or kidney, he should keep some of the following facts in mind. A tissue which is transilluminated absorbs light energy, and converts it to heat. Any structural element, whether it has visible color or not, will absorb some wave lengths to a greater extent than others. If the organ is very thin, the heat generated can be dissipated rapidly, and it is not so difficult to keep the tissue more nearly under physiological conditions. If, however, the organ is thick, a great deal more light is absorbed, and it becomes much more difficult to conduct away the generated heat rapidly enough to keep from overheating the tissue locally.

Further, if a wash of Ringer's solution is used to conduct away the generated heat from the region under study, every attempt must be made, at least in mammals, to keep the entire organ or tissue exteriorized under conditions as physiological as possible. This ideal is not attainable, perhaps; for one thing, so-called "physiological" saline solutions are not the normal environment of viscera. There is rapid exchange of electrolytes between the wash and the interior of an organ. One bit of evidence of this fact is the speed with which adrenaline or histamine added to the wash solution affects the circulation within the organ. We have tried a neutral paraffin oil bath, but this does not conduct heat away from the tissue rapidly enough, and local overheating results immediately, with resultant stasis or other serious traumatic effects.

A thick piece of tissue also requires a much more intense light in order to be transilluminated. The effect of strong light upon an organ, which normally never is subjected to

visible radiation, may be much greater than we realize. In a few cases, we can see directly some of these effects, such as vasoconstriction or vasodilatation, but we do not know all of them.

Another factor which must be taken into account is the problem of anesthesia. In the study of thin external tissues, such as the ear, or tadpole's tail, no anesthetic is needed. But an internal organ cannot be studied without the benefit of anesthesia. It is sometimes difficult to evaluate the effect of an anesthetic upon the local capillary circulation of an organ. We have attempted at times to control this question by comparing a local circulation under anesthesia with the same circulation in the unanesthetized animal. This can be done most easily on the rabbit's ear, or web of the frog's foot, or a similar tissue. Ether, for example, frequently produces pronounced changes in capillary circulation, and the barbiturates sometimes do.

METHODS

In the previous paper on the circulation in the spleen (Peck and Hoerr, '50) we outlined the principle features of exteriorizing the mouse spleen for transillumination. In the investigation reported here, we added a side-neck to the tube delivering Ringer's solution to the spleen suspension tray. The side-neck was connected with a second reservoir of Ringer's solution at room temperature. The constant temperature bath for heating the original supply of Ringer's solution was now maintained at a higher temperature than usual. The mixed flow from the two sources of saline solution could now be regulated by screw clamps so as to supply the spleen tray with Ringer's fluid at any desired temperature from room temperature to 43° C. Changes in temperature could be made either rapidly, or so slowly as to raise or lower the temperature of the Ringer's fluid only a degree in an hour.

The capacity of the spleen tray was 20 to 30 cm^3. The fluid was changed at rates of from 5 to 20 cm^3 per minute.

Even the minimum flow exchanged the fluid around the spleen with sufficient rapidity, so that not only was the entire spleen maintained at the desired temperature, but the temperature was uniform throughout the tray. This fact was determined by placing a two-inch thermometer, accurately calibrated for total immersion, in different parts of the tray, close to the spleen and along the sides.

In some experiments, there was some leakage of Ringer's solution from the tray into the peritoneal cavity of the mouse, but when we had learned to make the incision as small as possible, this leakage was minimal in the later experiments. Therefore, although the spleen might be suspended in solutions of different temperatures, there was little effect of temperature changes on the abdominal cavity of the mouse. The mouse itself, exclusive of its spleen, could be kept dry and warm at the room temperatures, which ranged from 72° F. in winter to 85° F. in the summer.

On one series of 30 mice, the following routine was established. The spleen was maintained for 15 to 60 minutes at 37.5° C., and the observed circulation of a selected region of the red pulp was considered as "standard" for that animal at that time. Then the temperature of the suspension fluid was raised, over a period of 5 to 50 minutes in most cases, to 40.5° C. for at least 10 to 30 minutes, and then lowered to 37.5° C. When the circulation had returned to that originally observed, the temperature of the Ringer's solution was then lowered to 34° C. It was maintained at 34° C. or 35° C. for 10 to 30 minutes, and then raised once more to 37.5° C. If, now, the circulation returned once more to the original pattern of activity, we assumed that the spleen had not been injured by the experiment.

In 10 experiments, the above procedure was repeated three or 4 times; if essentially the same temperature change effects occurred in identical manner, we concluded that the vascular changes were caused primarily by the changes in temperature, and not by one of the other variables of the experiment, such as changes in light intensity. Other experi-

ments were made to study the effects of quite sudden, momentary shifts in temperature, or, particularly in the earlier experiments, to study effects of extremely slow changes by changing the thermostat of the constant temperature bath. In some of the mice the spleen was maintained at a selected temperature for an hour or longer, to study the effect of long maintenance at temperatures varying from the normal. In a few mice, the temperature was first lowered to 34° C., to rule out any permanent effects of first raising the temperature above 37.5° C.

Usually a chosen field was studied almost continuously, alternating from low to high magnification, with an occasional shift to a neighboring field merely to see whether the circulation was uniform throughout the spleen.

OBSERVATIONS

Increase in temperature

The circulation at 37.5° C. has been described in the previous paper. A pattern observed in a particular mouse was assumed to be the norm. Spleens vary in activity at different times, some having rapid storage cycles in the venous sinuses, others having prolonged storage phases. Among the factors we know to be causative, we may mention the length of time since the last feeding. The "standard" pattern of activity usually included some venous sinuses in storage phases, others in continuous filtration phases. Those in storage phase were watched until they had undergone several emptying and filter-filling cycles or it was felt they were in a long duration phase.

As the temperature was raised from 37.5° C. to 40.5° C. the spleen became smaller and lighter in color. Under low power, the patches of red decreased, and the white areas increased until the spleen was quite pale with thin interlacing bands of red. Whether, in any individual experiment, we began with a relatively dilated or a somewhat contracted spleen, the

result of raising the temperature was always contraction and paling of the spleen.

Under high power the following changes were usually observed. The blood flow in the arterioles increased slightly in speed. A greater number of capillaries became visible in the selected field, with a rapid and steady blood flow. Sinuses which had been in the storage phase at 37.5° C. began to empty one by one. Sinuses which had been conducting blood went into storage phases for only a few seconds, if their activity changed at all. As the highest temperature was reached, there were usually still a few sinuses in the storage phase.

In the venules, the blood flow and concentration of cells varied with the activities of the sinuses. A venule draining an emptying sinus would have a great concentration of blood cells for a short time. A venule receiving blood from a "capillary shunt" would have a more constant and rapid blood flow. The blood flow in the veins increased greatly in amount for short periods, at the times when a number of venous sinuses emptied their previously stored blood cells. Then the concentration of cells decreased in the venous blood, the veins decreased slightly in diameter, and the blood flow, although not so concentrated in cells, became more rapid.

Maintenance of the spleen at 40.5° C.

Under low magnification, the spleen was small and pale. Splenic contractions occurred more frequently than at lower temperatures. The ratio of white areas in the spleen to red areas was high.

The arteriolar blood flow, although usually rapid and constant, was erratic. The blood flow in a particular arteriole would often cease for several minutes. The diameter of the arterioles was less than at 37.5° C. A smaller arteriolar diameter is the rule in contracted spleens.

The apparent number of capillaries was greatly increased. Rapid blood flow was observed for long periods in capillaries which at 37° C. were only occasionally visible. Splenic con-

tractions caused many of the capillaries to "shut off" momentarily.

Only a few venous sinuses remained in the storage phase. Most of them emptied before the temperature of 40.5° C. was reached. If a temperature of 40.5° C. was maintained for 20 to 60 minutes, more and more of the sinuses emptied and began the conducting phase. Occasional sinuses began continuous filtering phases, but above the temperature of 39° C. no sinus was seen to change from a conducting phase to a storage phase. The blood flow in the venules and veins was usually rapid, and the concentration of red cells in the venous blood, as compared with flow seen at lower temperatures, was low.

Decrease of temperature

As the temperature of the Ringer's solution surrounding the spleen was slowly lowered, the spleen became darker, and under low magnification the ratio of white areas to red areas decreased.

The number of "visible" capillaries, that is capillaries with blood flowing in them, decreased. Some venous sinuses began storage phases soon after the temperature began to go down. The lower the temperature became, the greater was the number of sinuses packed with red cells. A number of venous sinuses would fill with red cells, begin to pack them closely, rapidly empty, and then repeat the cycle. As the temperature decreased, the storage periods became longer.

As more and more sinuses went into the storage phase, the blood flow in their afferent capillaries ceased until the sinuses emptied. Blood flow, however, continued to be rapid in the capillary shunts. Many of the venules had a very low concentration of red cells in their blood, indicating that the flow was largely plasma.

Maintenance of the spleen at 34° C.

At a temperature of 34° C. maintained for 10 minutes to an hour, the spleen was dark red. The splenic contractions were infrequent, but pronounced.

Practically the only capillaries with an active blood flow were those connecting arterioles and venules, the capillary shunts. Nearly all the venous sinuses were in the storage phase, some emptying partially and briefly, immediately to pack and store cells again.

The veins were dilated. The blood flow was sluggish, irregular, variable in concentration of cells, and often showed complete reversal in direction.

Return of the spleen temperature to 37.5° C.

Depending upon the length of time the spleen had been maintained at a temperature of 34° or 35° C., the circulation returned to the original pattern of activity more or less rapidly when returned to 37.5° C. If the spleen had been kept at 34° C. for an hour, the "standard" activity returned only after a latent period of 30 minutes to an hour.

The degree and rapidity of change in the splenic circulation in any one spleen was dependent upon the original activity seen when the spleen was exteriorized. Shortly after a meal, the mouse spleen tends to be expanded, and contains a high proportion of venous sinuses in the storage phase. These dilated spleens reacted most slowly to temperature changes, and always contained a certain number of sinuses in the storage phase, even at a temperature of 40.5° C.

On the other hand, a spleen which was contracted when first exteriorized, would contract still more as the temperature was raised, and would store more red cells when the temperature was lowered. The partially contracted spleens reacted to thermal changes more rapidly and to a greater degree.

Rapid changes in temperature

A rapid increase in temperature caused a strong splenic contraction. This was followed by a dilatation, and then by frequent contractions until the spleen exhibited the pattern characteristic of the higher temperature. In general, the venous sinuses and venules emptied almost completely with

the first strong contraction, and thenceforth conducted whole blood at a rapid rate. The sinuses did not return to storage phases.

A rapid decrease in temperature also caused a strong contraction of the spleen, after which the spleen would dilate, and then contract at infrequent intervals, exhibiting the sinuses predominantly in the storage phase.

Summary of thermal effects upon the circulation

In general, it can be said that a rise in the environmental temperature converts the spleen from an organ of red blood cell storage to an organ in which the blood flow, similar to that in many other regions of the body, is relatively constant and rapid. Conversely, as the environmental temperature is decreased, there is increased storage of red cells. The degree of sequestration of cells from the circulating blood is directly proportional to the lowering of the temperature, within the limits investigated.

The vessels influenced most by thermal changes were the capillaries, venous sinuses, and venules. Little change in the arteriolar blood flow was observed. The arterioles constrict, dilate, stop and start blood flow at intervals which seem to be largely independent of temperature changes or of the degree of contraction or expansion of the spleen. However, the arteriolar flow is more erratic at higher temperatures, and the arteriolar diameters tend to be less in contracted spleens.

The flow of blood in the veins seems to be entirely dependent upon the activities of their afferent vessels. In a spleen exhibiting a high degree of storage, constant, rapid blood flow is seen primarily in the capillary shunts directly connecting arterioles with venules. In a contracted and overheated spleen nearly all the capillaries and venous sinuses conduct whole blood rapidly.

Repetition of the MacKenzie experimental set-up

MacKenzie's spleen tray, as described in his paper, photographed in his figure 1 (p. 401), and drawn in his figure 2

(p. 403), was duplicated exactly. Briefly, MacKenzie's experimental set-up was as follows. The spleen was drawn through a hole in a horizontal celluloid strip, and supported on this strip by absorbent cotton wadding. The spleen was surrounded with cotton which was flooded with Ringer's solution. A cover slip was placed on this cotton "fence" over the spleen but not touching it. A laboratory thermometer was placed alongside the cotton wall surrounding the spleen, and the bulb covered with cotton. The celluloid platform supporting the spleen was partially converted into a tray or chamber by the addition of several side walls. The tray was not completely enclosed, however, so that the Ringer's solution, brought to the spleen compartment by a delivery tube and the hollow-tipped quartz rod, dripped over the edge of the celluloid platform.

We exteriorized and suspended mouse spleens in the tray exactly according to MacKenzie's description. The circulation in the red pulp was observed to be fairly constant and rapid. There were few storage phases, and almost no sphincter action, in the venous sinuses. Splenic contractions occurred at frequent intervals. Although, at first glance, the blood flow through the red pulp seemed to be an aimless wandering of red blood cells through a swamp, we were able to distinguish vessels. As we have described in a previous paper, by trying to eliminate all sources of minute vibration, we succeeded in seeing intact, continuous, smooth, thin, refractile walls in arterioles, capillaries, venous sinuses and venules.

We then checked the temperature of the Ringer's solution immediately surrounding the spleen with a small thermometer, two inches in length, with a bulb 5 mm long. One of these thermometers, originally calibrated for total immersion, was re-calibrated for bulb immersion only.

When the large laboratory thermometer read 38° C. the small thermometer with its bulb touching the spleen read a fraction of a degree over 40° C. The thermometers had previously been checked against each other.

Repetition of the Knisely experimental set-up

Knisely exteriorized only the lower pole of the spleen and did not attempt to support it on a tray. He merely stuck the tip of the quartz rod under an edge of the spleen, as illustrated in his figure 1 (Knisely, '36, p. 27). Although he does not say so in his paper, he tells us that he placed wisps of non-absorbent cotton on the mouse around the spleen forming a drainage trench to keep the Ringer's wash off the animal's body as much as possible.

It was not possible for us, in duplicating Knisely's procedure as closely as possible, to determine accurately the temperature of the environment of the spleen. The wash Ringer's in some instances was allowed to drip off the immersion lens of the microscope and flow over the spleen. This covers the spleen with a thin film of constantly renewed Ringer's fluid. Since this film is subject to rapid cooling by radiation and evaporation, our small thermometer, with the bulb wrapped in absorbent cotton and placed against the spleen or under its lower pole, sometimes gave readings of 36° C. instead of 37.5° C. or 38° C.

In general, the circulatory pattern seen in this set-up is essentially the same as described by Knisely. The spleen is difficult to observe, as very little attempt can be made to immobilize it from respiratory movements of the animal. Also the storage cycles of the venous sinuses tend to be more prolonged than in a spleen studied in the tray we devised, in which the temperature of the suspending Ringer's solution can be maintained accurately at 37.5° C.

DISCUSSION

On one point, Knisely and MacKenzie seem to agree, the contraction of the spleen with a change in temperature. Knisely says, ('36, p. 134),

"This same set of changes, namely, clamping of spincters and passage of red cells into pulp partitions, occurs whether the stimulation consists of cutting, pinching, scratching, stretching, or twisting the spleen, the gastrolineal ligament, or the vessels and nerves at the

hilus. These reactions occur if the spleen is touched with perspiring finger tips or with metallic instruments which are at room temperature. Similar reactions occur, but more slowly, if the temperature of the Ringer's wash solution varies more than 1.5° C. from the temperature of the abdominal cavity of the animal."

MacKenzie states (p. 436),

"*Effects of local temperature changes.* By introducing small quantities of hot or cold Ringer-Locke solution through a burette into into the chamber delivery tube, it was possible to produce slight, brief temperature changes in the fluid environment of the exteriorized spleen. We found that a sudden fall, of as little as 1.5° C., caused a definite, if fleetingly transient paling of the red pulp, associated with irregular, jerky flow in the arterial capillaries and elimination of many of the intercellular channels that had previously conducted blood: in other words, that splenic contraction and arterial constriction were produced. Raising the chamber temperature several degrees evoked similar effects. In the general course of our experiments, it was noted that the most obviously 'open' circulations were present in those specimens for which a chamber temperature of 38° C. was constantly maintained."

We likewise observed that a sudden temperature change, either a raising or lowering of the temperature, caused an immediate pronounced contraction of the spleen.

From the fact that MacKenzie reported little storage of red cells, no sphincter action in the red pulp, and primarily a constant and rapid flow of blood through the spleen, we suspected that he might have maintained his spleens at higher temperatures than 38° C. as he thought. The activities he described fit most closely our observations of circulation in spleens maintained at 40.5° C.

The large laboratory thermometer such as MacKenzie used is usually calibrated for two-inch or 76 mm immersion. If only the bulb is immersed it will give a lower reading than the true temperature. Also, as we have stated, there is usually about a two degree drop in temperature, in MacKenzie's type of set-up, from the spleen to the thermometer outside the absorbent cotton wall around the spleen.

With absorbent cotton forming a small compartment around the spleen, furthermore, there may not be sufficient Ringer's solution, or a sufficiently rapid rate of change of the wash, to conduct away from the spleen all the heat generated by the absorption of light. Also, we suspect that the mouse was not kept dry. The photograph (fig. 1, p. 401) gives one the impression that the belly fur of the mouse had been wetted by the drainage of the wash fluid. The chamber, of course, did not have complete walls.

Conversely, we suspected that Knisely was maintaining his exteriorized splenic tips at lower than 37.5° C., since at this temperature the storage cycles are not so prolonged nor so frequent as they are at 36° C. or lower. Unless great care is taken to secure precise temperature control, it is very easy, with Knisely's set-up, to maintain the spleen at varying temperatures in different parts, and especially to obtain lower temperatures than suspected. Knisely has discussed thoroughly this problem of temperature control in his 1937 paper.

Of course, we have no knowledge of the circulation of the normal spleen in the intact peritoneal cavity. However, the experiments reported here, we believe, explain some of the differences in the observations of Knisely and MacKenzie. The *exteriorized* spleen reacts with great sensitivity to environmental thermal changes.

CONCLUSIONS

1. The environmental temperature of the exteriorized spleen can be maintained precisely only with the aid of a completely walled suspension tray of sufficient capacity to conduct away heat generated by absorption of light.

2. Raising the temperature of the Ringer's solution bathing the spleen to 40.5° C. eliminates the storage phases of the venous sinuses, causes the circulation of the spleen to become constant and rapid, and abolishes all sphincter actions except those of arteries and arterioles. This pattern corresponds with that described by MacKenzie, Whipple and Wintersteiner, '41.

3. Lowering the environmental temperature of the spleen below 37.5° C. increases the red blood cell storage activity of the spleen. The lower the temperature, the more prolonged and more predominant are the storage phases of the venous sinuses.

4. Temperature changes have more effect upon the capillaries, venous sinuses, and venules of the red pulp than do spontaneously occurring contractions and dilatations of the spleen.

5. Sudden thermal changes, both raising and lowering the temperature, cause a strong initial contraction of the spleen.

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THE DEVELOPMENT OF THE EFFERENT ARTERIOLE IN THE HUMAN METANEPHROS

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TWO FIGURES

INTRODUCTION

During the 108 years since Boman (1842) discovered the relation of the renal glomerulus to the nephron, very little beyond surmise has been done concerning what happens after the afferent arteriolar branches give rise to the glomerular capillaries. Ludwig (1872) gave an interesting account of certain sequences in glomerular development. He held that each afferent arteriole entered a spherical dilation at one end of a developing nephron and divided into from four to eight branches. From these branches, within the glomerular capsule, a "brush of free capillaries" arose which constituted a glomerulus. These "free capillaries" in due course "coallesce" to form the efferent arteriole. Sappey (1879), on the basis of his own observations, gave a description similar to that of Ludwig. He stated that the vas afferens penetrated the glomerular capsule and divided at once into several branches which, after dividing and subdividing, gave rise to non-anastomosing capillaries. These developed into loops and became continuous with the first "roots" of the efferent arteriole. The latter was formed by the successive reunion of several of such roots and then left the glomerulus close to the place where the afferent arteriole entered.

Despite these accounts which clearly indicated a relatively late development of the efferent arteriole, Huber's ('05)

figure 8, page 50, included diagrams of glomerular development which show two vessels present from the beginning of such development. This figure, regardless of patent glomerular incorrectness, continues to be reproduced in textbooks of embryology without pertinent comment by their authors. Interesting here is the fact that Felix ('12) and Shikinami ('26), who presumably paid some attention, however incidental, to stages in glomerular development in connection with their study and depiction of the development of the human mesonephric tubule, did not depict any aspect of glomerular development in their figures 561, p. 804 and 13, plate 7, respectively. They merely outlined the area where glomerular development would occur. Authors of textbooks of embryology in which Felix and Shikinami's figures have been reproduced, might better have left them unmodified than to have supplied misleading pseudoglomeruli in the glomerular area as outlined by Felix and Shikinami.

It is strange that among those investigators who studied glomerular development, none appears to have been concerned with the development of the efferent arteriole. Notable among such investigators are Ribbert (1880), Herring ('00) and Vimtrup ('26). They studied afferent arterioles and glomerular capillaries in various stages of glomerular development, but apparently did not study adequately such stages as would have revealed the development of the efferent arteriole.

MATERIAL AND RESULTS

Pieces of the kidneys of an eighteen weeks old fetus; of two stillborn term fetuses, and of one newborn infant were studied in serial sections. It was noted in a preliminary study that at the junction of the afferent arteriole with one or more of its first branchings there is frequently a dilatation designated by certain German investigators as a glomerular sinus. The site of the union or fusion of groups of glomerular capillaries to form the beginnings of the efferent arteriole is not markedly characterized by a sinus-like dilatation.

Glomerular development begins at the cortico-medullary junction and then proceeds peripherally in a wave-like manner. The medullary parenchyma, therefore, is the first to become vascularized. At birth, this parenchyma and that of a narrow zone bordering it, are the only parts having a capillary and thus a functional blood supply. The efferent arterioles develop only after the glomeruli have attained a size almost as great as those that have become functional (fig. 1, A, B, C), namely, 65-75 μ in diameter. At the time of their first appearance along the cortico-medullary junction, the peripheral two-thirds or more of the cortex seems to have a normal blood supply, but a microscopic study reveals that whereas all renal arteries, veins and glomeruli in this portion of the cortex contain blood, there is none around the tubules. The reason for this consists in the fact that the efferent arterioles are still blindly ending cords of cells without lumens (fig. 1, D, E).

It may be appropriate here to comment that studies on the functioning of the fetal or early postnatal metanephros must apply chiefly to nephrons located partly in the cortico-medullary zone and, via their loops, partly in the medulla. Also, it is possible that this first established medullary circulation is relatable to the Trueta ('47) by-pass or shunt as well as to the fact that the vessels of the medulla in the injected kidney, rarely fail to be completely injected, while those in the cortex proper are not. However this may be, the fact remains that the efferent arterioles are the last non-capillary vessels to develop in the kidney. The peritubular capillaries develop later from them either directly in the cortex proper (fig. 1, F) or indirectly from branches of the arteriole rectae in the medulla (fig. 2). The efferent arterioles which descend into the medulla are in the late fetal kidney as well as in that of the adult, about twice as long as those located in the cortex proper. Their measured lengths in the kidney of the newborn are 60–100 μ in the cortex and 150–200 μ for those descending into the medulla. Those arising from the glomeruli located in the cortico-medullary zone but which do not descend into

the medulla are short like those arising from glomeruli located in the cortex proper.

Glomeruli 60–70 μ in diameter and whose capillaries are filled with blood, have efferent arterioles composed of solid cords of cells which vary in length depending on their location in the cortex. Lumen formation in them usually does not begin until they have developed capillary sprouts (cortex) or initial branchings to form arteriolae rectae (medulla). Luminal development proceeds distally from the glomeruli. Occasionally, such development starts in the mid-portion of the arteriole and reaches its glomerular and distal end at about the same time. When the efferent arteriolar lumen reaches its cortical capillary or medullary arteriolar end, blood is present in it. This is in contrast to the afferent arteriole whose lumen and the lumens of the capillaries arising from it, are filled with blood from the beginning of their development. Thus, there must be a period of glomerular stasis before the efferent arterioles become functional.

SUMMARY

Efferent arterioles in the fetal human kidney arise only after the afferent arterioles and glomerular capillaries have completed their development. They begin as solid cords of cells each of which later acquires a lumen. The first to appear arise from glomeruli located along the cortico-medullary junction and 80–85% descend into the medulla. They are

Fig. 1 Diagrammatic representation of the development of the glomerulus and efferent arteriole. (A) Afferent arteriole in an early relation to the nephron. (B) Initial branches of the afferent arteriole from which glomerular capillaries arise. The inner layer of the glomerular capsule is shown investing these branches and a histological section of it is indicated by the arrow. (C) Some of the glomerular capillaries converging to form an initial beginning of the efferent arteriole. A segment of the inner capsule layer is indicated by the arrow. (D) Definitive efferent arteriole, upper right, shown emerging from the glomerulus. (E) Further growth of the efferent arteriole. It is still a cord of cells without a lumen. The inner (visceral) layer of the capsule as indicated by the arrow has assumed its mature structure. (F) The fully developed efferent arteriole showing a portion of the cortical capillary plexus which proliferated from it.

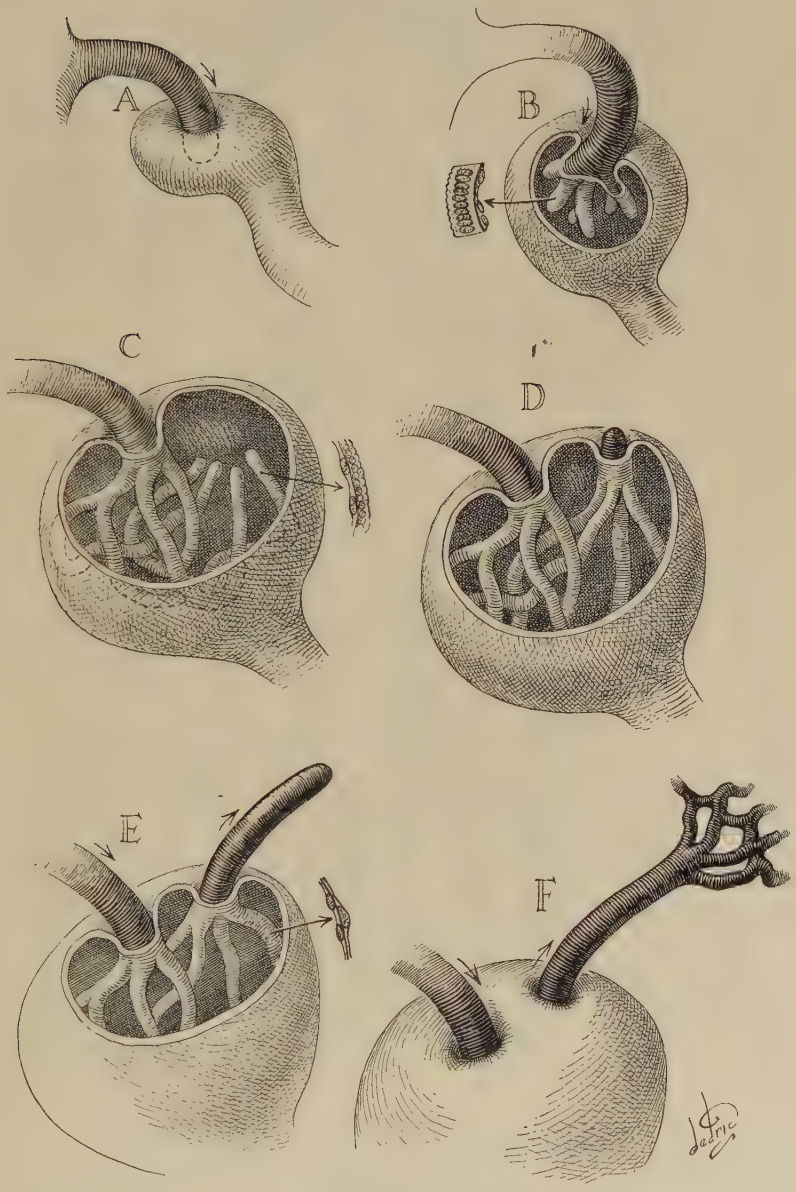

Figure 1

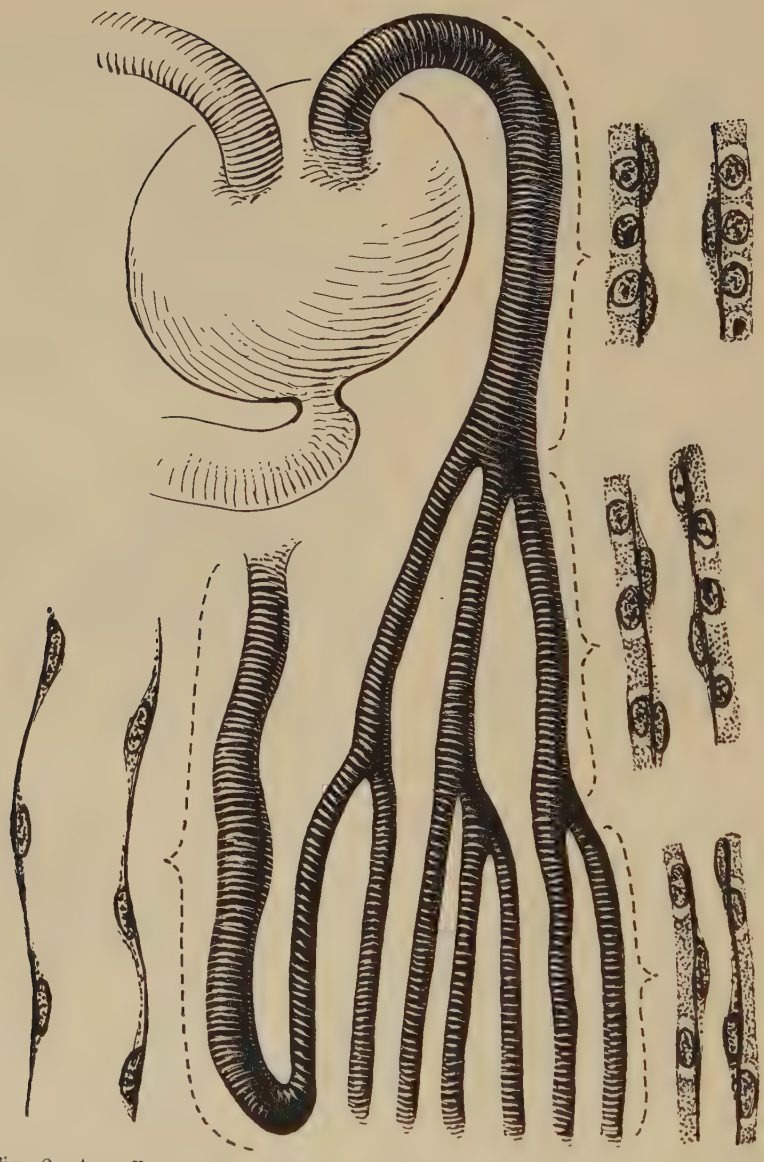

Fig. 2 An efferent arteriole typical of those descending into the medulla. Note its 3 primary and 7 secondary muscular branches (arteriolae rectae) and at the right of the brackets the character of their walls. At the lower left on either side of the bracket is shown a venula recta with which a secondary arteriola recta is continuous. The so-called venulae rectae compose the medullary capillaries which terminate by joining the arcuate veins.

approximately twice as long as those arising later in the cortex proper. In the kidney of an adult they are 3–4 times as long. Arteriolae rectae in the medulla and peritubular capillaries in the cortex, begin their development from the distal end of the efferent arteriole before the latter acquires a lumen. The efferent arterioles and arteriolae rectae have each a distinguishable single layer of smooth muscle and an endothelial lining soon after or coincident with their acquisition of a lumen.

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THE FUNCTION OF NERVE FIBERS WHOSE STRUCTURE HAS BEEN DISORGANIZED

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FIFTEEN FIGURES

The vast literature devoted to nerve fiber conduction contains many references to (a) the relationship which exists between the conducting properties of a fiber and such morphological features as its dimensions and degree of myelination and (b) modifications of the conducting properties which are brought about as the result of deviations from the normal morphological condition of the fiber. From such observations has emerged the belief, which is now widely accepted, that any deviation from the normal structure of a nerve fiber is associated with some impairment of its functional efficiency.

The object of this paper is to report observations made during the course of an investigation into the relative merits of different suture materials for the repair of nerves. These indicate that nerve fibers may continue to function efficiently after they have been so modified architecturally that their sheaths cannot be identified and they can no longer be recognized as myelinated fibers.

MATERIAL AND METHODS

Thirty-nine experiments (details of which have been provided elsewhere, Sunderland and Smith, '50) were performed in which the median and ulnar nerves and several cutaneous nerves were transfixed with catgut, chromic gut, silk, human

[1] This work was assisted by a grant from the National Health and Medical Research Council of Australia.

hair, tantalum and nylon sutures. The common Australian opossum, Trichosurus vulpecula, was used for the experiments. Under chloroform anaesthesia the nerve was exposed under aseptic conditions. The hair was removed and the skin prepared with iodine. Following the skin incision only normal saline was used. A small round-bodied atraumatic needle was used for carrying the suture material which was drawn obliquely through the nerve and tied lightly so as to avoid destruction or constriction of the nerve. In some nerves the suture was passed through the funiculi and in others between them.

The wounds were closed, swabbed with iodine and covered with collodion. With one exception they healed by primary intention. The animals remained healthy throughout the investigation and none lost weight. They were allowed the full freedom of a large cage so that their normal activities were unrestricted.

Twenty-nine of the experiments were terminated after an interval of 21 days while in a second group of 10 the involved area was left undisturbed for 117 days. The nerves were then exposed, the local reaction studied and a segment of nerve containing the involved area removed. After fixation in 10% formalin the excised segment was subjected to a variety of staining techniques for the purpose of histological examination of the various tissues comprising the nerve.

Transverse sections of each nerve in the uninvolved zones above and below the suture together with sections of the segment of the nerve at the site of maximal reaction were enlarged 25 times on a microprojector and drawn. The cross-sectional area of the nerve and its component funiculi were measured on these diagrams by means of a planimeter. From cross-sectional analyses of the nerve at these three levels it was often possible to express metrically the extent of the reaction and to estimate any reduction in the cross-sectional area of bundles which had been involved at the site of implantation.

Representative muscles innervated by the nerves selected for investigation were removed, weighed and examined histologically for signs of denervation atrophy.

OBSERVATIONS

1. In the experiments on the median and ulnar nerves no signs of motor disability could be detected during the entire post-operative period and the animals used the involved limbs quite normally. This suggested that injury to nerve fibers when inserting the suture or as a result of the reaction to the implanted suture material had been negligible. This was confirmed by histological examination of representative muscles taken at autopsy from the fields of the involved nerves.

2. Implantation of the suture materials, particularly the silk, catgut, and nylon, was followed by a massive cellular and fibroblastic reaction so that the nerve was swollen to several times its original size at the site where the suture had been inserted. Details relating to the nature of this reaction and the cell types participating after the insertion of the various suture materials commonly employed for the repair of severed nerves have been reported elsewhere (Sunderland and Smith, '50). The significant observation with which the present paper is concerned relates to funicular and nerve fiber picture at the site of the maximal reaction and at levels distal to this.

When serial sections of the nerves were examined from above downwards it was found in many specimens that, as the swollen site of implantation was approached and the intensity of the reaction increased, levels were reached where all traces of the funiculi were lost (figs. 1 to 15). The histological picture was one of a suture surrounded by a dense cellular mass in which the funicular outlines were completely obliterated and in which no nerve fibers could be identified. No myelin could be detected and though Schwann cells may have been related to the axones they were not organized in a manner suggestive of a continuous linear sheath. Further experiments are in progress to determine the fate of the myelin

and the Schwann cells in the amorphous mass of cells and fibers.

Despite the complete disorganization of the intraneural architecture which occurred at the site of implantation, transverse sections of the nerve at more distal levels revealed a totally unexpected picture. Aftr emerging from the involved area the nerve had its normal funicular appearance restored, the bundles showing few traces of the reaction which had obliterated them at a slightly higher level (figs. 1 to 15).

This picture was observed when the suture was implanted between the bundles as well as when a funiculus or funiculi had been transfixed. Whether or not the funiculi were submerged in this way depended on the suture material employed and the size of the bundles comprising the nerve. Small bundles were more commonly obliterated than the single large funiculi comprising such nerves as the ulnar and the median which nevertheless provided several examples in which all traces of the funiculus were lost.

3. Though in these specimens the funicular outlines were completely obliterated by the reaction and the structure of the nerve fibers so modified that they could no longer be identified by their characteristic morphological appearance, the following evidence suggests that the majority of these nerve fibers survived and functioned efficiently.

I. The axones of the majority of the fibers, though dispersed by and intermingled with the proliferating fibroblastic and cellular elements and deprived of their normal sheaths, continued unharmed through the mass. Distally they emerged to regain their sheaths and to present a structure which, by the staining methods employed, appeared normal. The morphological disorganization of nerve fibers described involved only short segments of the fibers — no longer than 7 mm.

II. Wallerian degeneration was much less than was anticipated from the conditions obtaining at the site of implantation. There was also very little Schwann cell activity distal to the site of involvement. Had the bulk of the fibers sustained an injury leading to Wallerian degeneration such activity should

have been well in evidence 21 days after the implantation of the suture. The extent and disposition of the Wallerian degeneration and Schwann cell activity indicated that the only fibers damaged were those directly traumatized by the needle and suture or those which had herniated through breaches in the perineurium created by the passage of the needle through the bundle.

III. The cross-sectional area of the bundles below the site of involvement was unchanged or only slightly reduced in comparison with that obtaining at proximal levels. Other experiments have shown that the funicular cross-sectional area of the distal stump of a severed nerve which has been denervated for 117 days atrophies to about 40% of its original value (Sunderland and Bradley, '50a). This atrophy is due to degeneration of the contained nerve fibers and to the shrinkage of the endoneurial tubes whose sole contents are, ultimately, Schwann cells. Had large numbers of fibers undergone Wallerian degeneration some funicular atrophy should have been evident at 117 days. The possibility remains that regenerating axones may have reduced the shrinkage but even so it is unlikely that regeneration through such a disorganized area as that obtaining at the site of implantation would, at 117 days, be of a degree sufficient to restore the funicular cross-sectional area to its original value.

IV. Post-operatively the animals showed no disturbance of motor function which could be detected by careful observation. This indicated that at least the majority of the nerve fibers were functioning efficiently. Electrical studies of their conduction properties under these conditions will be undertaken when equipment becomes available. Sargent and Greenfield ('19) reported a "slight and transient" paralysis of the calf muscles after transfixing the sciatic nerve with suture materials which induced an intense reaction in the nerve.

V. Muscles in the field of supply of the involved nerves showed no wasting, which would have been evident 21 and 117 days after the injury, while histological examination of these revealed no changes indicative of denervation atrophy (Sunderland and Ray, '50)

All these findings pointed to minimal damage to the conducting components of those nerve fibers whose characteristic outlines had been effaced as they traversed the area of reaction. For these reasons an examination of the nerve at the site of involvement may give misleading information about the state of the funiculi and nerve fibers at more distal levels.

CONCLUSIONS AND DISCUSSION

Nerve fibers may be so modified architecturally by the reaction developing about suture materials implanted in peripheral nerves that their sheaths cannot be identified and they can no longer be recognized as myelinated fibers. However an examination of serial sections of the nerves revealed that, despite the complete disorganization of the intraneural architecture at the site of implantation, the normal appearance of the funiculi and nerve fibers was restored at more distal levels. Evidence is presented which indicates that the fibers continued to function efficiently even though they could not be identified by their characteristic morphological appearance at the site of implantation.

The morphological disorganization of nerve fibers described in these experiments involved only short segments of the fibers — no longer than 7 mm. It is, however, interesting that axones can be deprived of their sheaths for short distances without affecting the structure of the fiber at more distal levels and that these segments, moreover, conduct efficiently under conditions which must surely facilitate interference effects in adjacent axones.

There is other experimental evidence (Weiss, '41; Alexander, Woods and Weiss, '48; Duncan, '48; Sunderland and Bradley, '50b) to indicate that nerve pathways regarded, on the basis of their diameters and degree of myelination, as morphologically incomplete may nevertheless excite normal activity at the periphery. These findings suggest that the relationship between fiber diameter and degree of myelination on the one hand and fiber conduction on the other may not be as intimate as we have been led to believe.

A further point of interest is that in working with the pathology of peripheral nerve injury great care must be exercised when examining the biopsy material removed from the suture line of cases of so-called "failed repair." An examination of this tissue may give misleading information about the state of nerve fibers at the site of union unless stains to reveal the axones are employed. Unfavorable looking tissue may contain axones which are capable of functioning efficiently despite the fact that pathways possessing the characteristic morphological features of myelinated nerve fibers cannot be detected crossing the suture line.

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PLATE 1

EXPLANATION OF FIGURES

1 to 9 Series of transverse sections from an ulnar nerve in which chromic catgut had been implanted 21 days previously. Sections 1 to 8 have been reproduced at the same magnification. Section 9 is an enlargement of that portion of 8 which is adjacent to the intraneural arteriole illustrated. Sections 1 and 2 were taken proximal to the site of implantation; the latter shows the cellular reaction which had spread proximally as a perivascular cuffing. As the sections are traced to the site of involvement the reaction increases in intensity and extent until finally all trace of the major funiculus and fibers comprising the nerve is lost (figs. 3 and 4). Sections 5, 6, 7 and 8 were taken at progressively more distal levels. They illustrate the reappearance of the funiculus below the site of maximal involvement though this still shows traces of the reaction. Signs of the cellular reaction are lost at more distal levels until finally the bundle and the contained nerve fibers present a normal appearance (figs. 8 and 9).

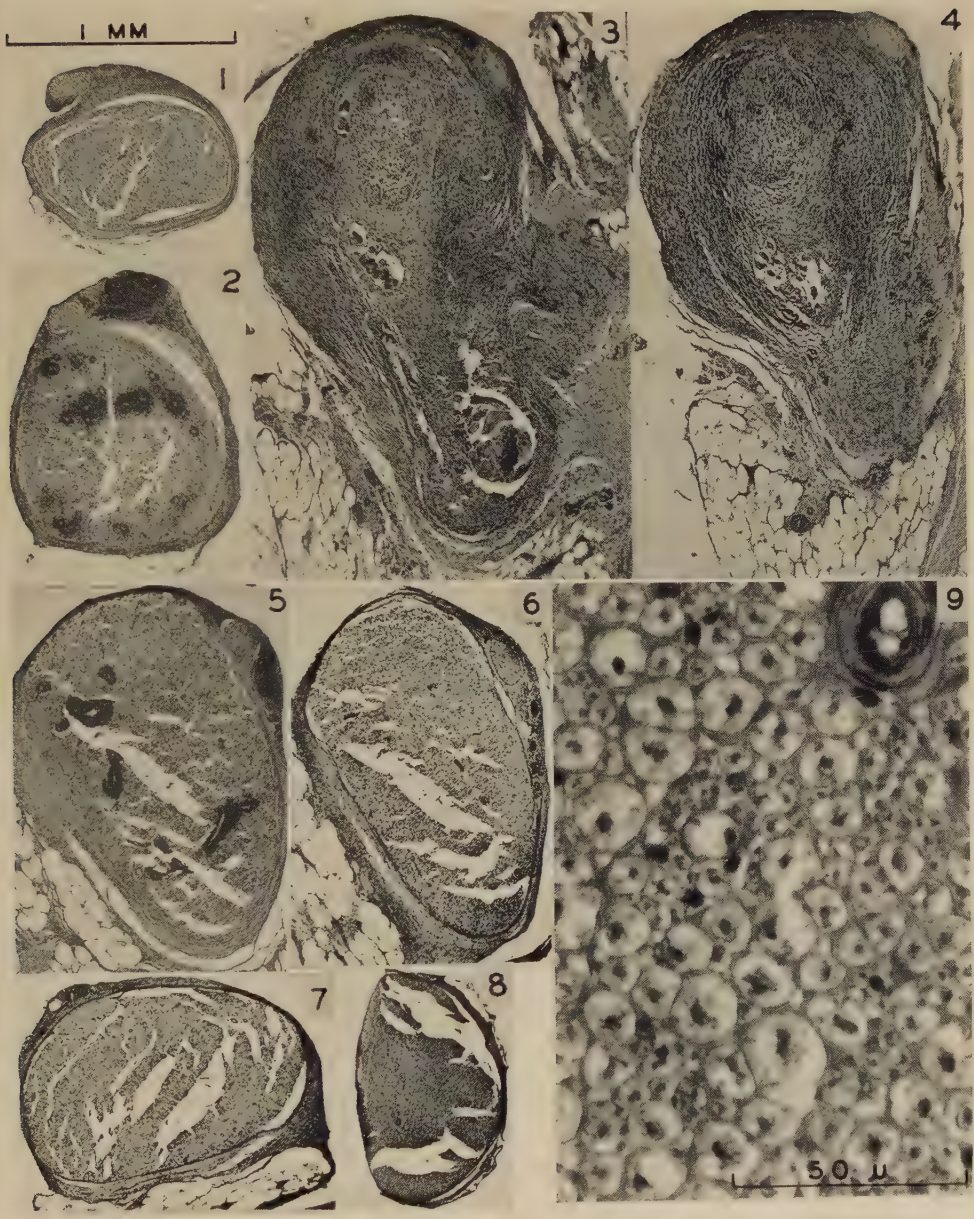
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PLATE 1

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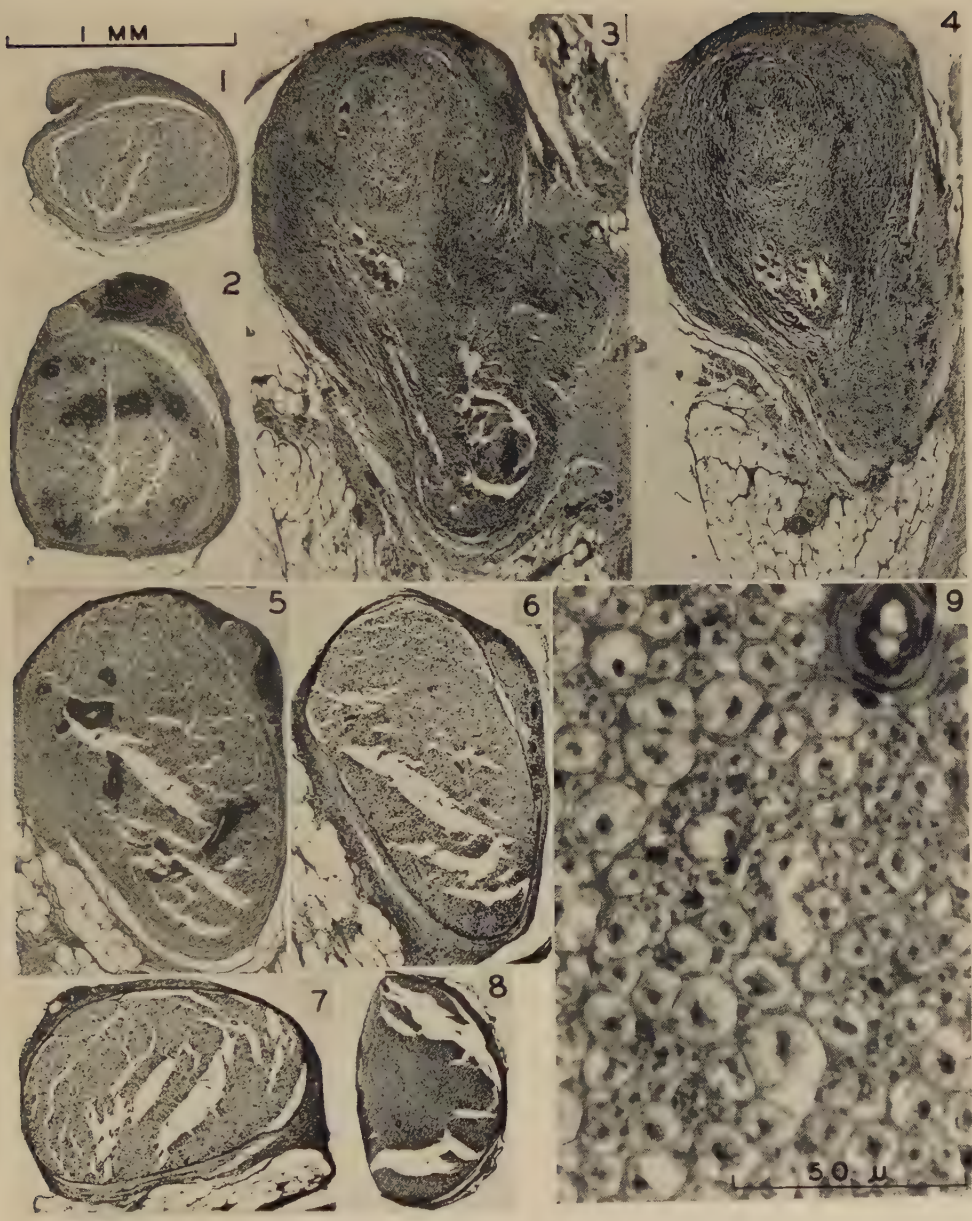
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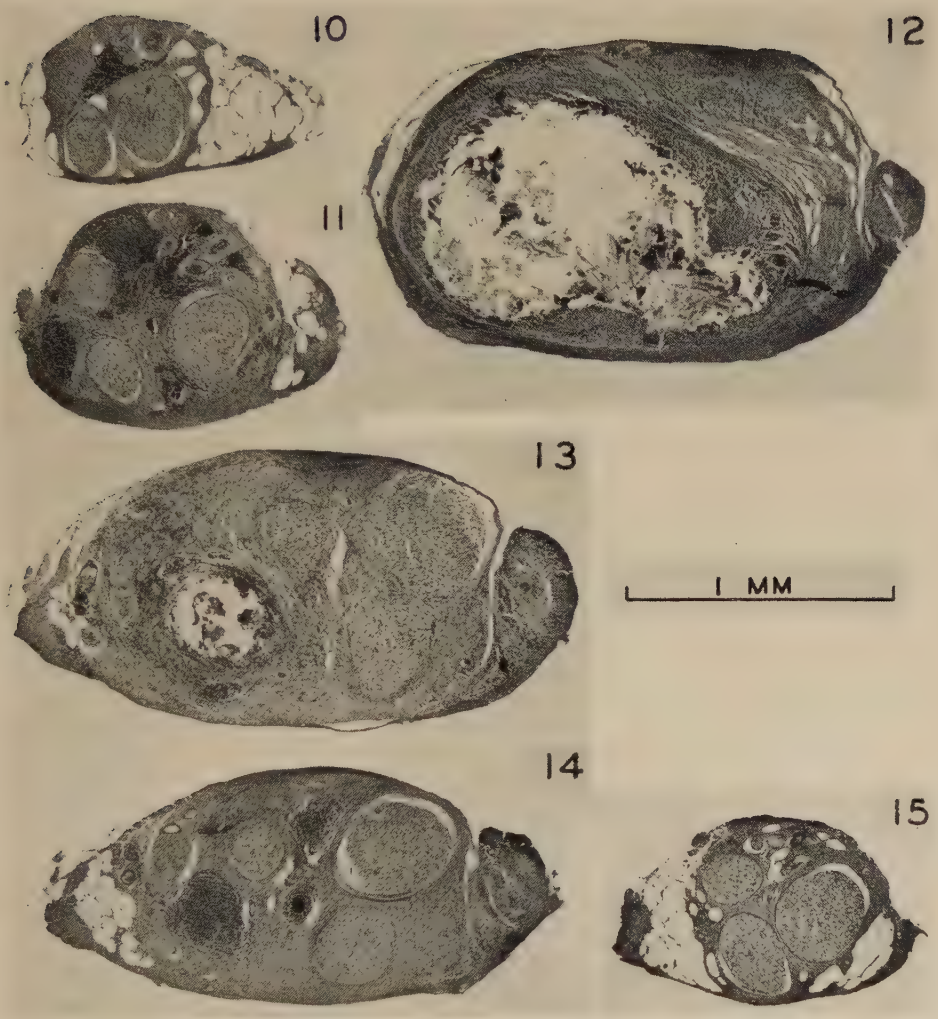
PLATE 2

EXPLANATION OF FIGURES

10 to 15 Series of transverse sections from a medial cutaneous nerve of the forearm in which white silk had been implanted 117 days previously. The suture material had been inserted through the nerve but between the component bundles. All sections have been reproduced at the same magnification. Sections 10 and 11 were taken proximal to the site of implantation and show the cellular reaction which had spread proximally to surround the bundles. Sections 12 and 13 were taken at the site of implantation; the bundles and nerve fibers can not be identified in figure 12 but are reappearing in figure 13. They are clearly defined in figure 14 in which evidence of the reaction is present in the interfunicular spaces. In section 15, the most distal of the series, the funicular appearance has been fully restored and there are few signs of the severe changes which had obliterated the bundles at higher levels. An examination of the nerve fibers and a comparison of the funicular cross-sectional areas in figures 10 and 15 indicate that the fibers had survived the changes at the site of implantation which rendered them unrecognizable.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EXPERIMENTS ON THE FATE OF THE LYMPHOCYTE [1]

RICHARD S. FARR [2]
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THIRTEEN FIGURES

INTRODUCTION

It is generally agreed that several billion lymphocytes enter the circulating blood each day via the thoracic and right lymphatic ducts (Rous, '08; Bunting and Huston, '21; Yoffey, '35–'36; Sanders et al., '40; Lawrence et al., '45; Adams et al., '45; Mann and Higgins, '50). Since the number of lymphocytes in the blood remains relatively constant under normal conditions, it follows that over a given period as many leave the circulation as enter it. In spite of the tremendous number of cells involved, it is not known where these lymphocytes that leave the peripheral blood are going although several hypotheses have been advanced to explain their disappearance. According to Bunting and Huston ('21), many of the lymphocytes migrate through the intestinal epithelium and leave the organism via the lumen of the intestine. Stenqvist ('34), however, found no histological evidence of this and he believes that the presence of lymphocytes in the lumen is a postmortal change. Sjövall ('36) explains the disappearance of lymphocytes from the circulation by the assumption that the lymphocytes eventually return to the lymphatic tissue. He believes that there is a "lymphocyte circulation in the organism: from the lymphatic tissue via the blood to the tissue out in the body and back to the place of origin." Yoffey and Drinker ('39)

[1] Aided by the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

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labeled cells. In the differential counts, monocytes and lymphocytes were classified together as mononuclear leukocytes.

To examine blood for the presence of labeled lymphocytes, observations were made on drops placed under a coverslip and ringed with paraffin. If labeled cells were not seen in such a preparation, the leukocytes were concentrated by centrifuging a sample of citrated blood and the buffy coat similarly examined. Tissues for histological studies were prepared by the Altmann-Gersh freezing-drying technique. The microscope was equipped with a turret condenser and both phase and conventional optical systems. Labeled nuclei in the tissues were located in the near ultra violet light with the conventional optics. By changing to the lenses for phase microscopy, while leaving the preparation in place, the cell could then be identified in white light.

Sections of tissue from untreated rabbits contain substances which fluoresce when stimulated with the light source used for these experiments. None of this naturally occurring fluorescence is intranuclear and most of it is of a color different from the fluorescence of 3,6,diamino-10-methylacridinium chloride. On this basis, the natural fluorescence and the diaminoacridine fluorescence can be distinguished. However, the natural fluorescence sometimes makes the observation of the labeled leukocytes difficult because of its relative brilliance. The most troublesome natural fluorescence is that of particulate inclusions seen in the cytoplasm of macrophages throughout the animal.

OBSERVATIONS

The effect of the operative procedure and the injection of the labeled lymphocyte suspension upon the peripheral leukocyte population of the rabbit was studied on a group of 18 rabbits. Total and differential leukocyte counts were made on each animal prior to anesthesia, 15 minutes before and after the injection of the cell suspension, and one, two and three hours after the injection. The combined results of these counts are presented in figure 1. It can be seen that the leukocyte

changes are not significantly different from those observed in rabbits after the intravenous injection of pyrogen-free saline.

Twenty-six rabbits were studied in order to determine how long the labeled lymphocytes remain in the circulation. It was technically difficult to quantify accurately the rate of disappearance because the number of labeled cells involved was relatively small. In addition, the thickness of the counting chamber considerably exceeds the working distance of the condenser, making it impossible to obtain enough light to

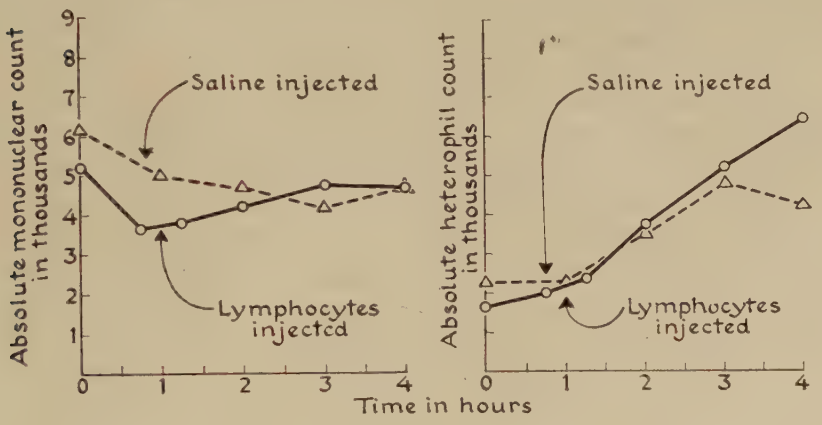

Fig. 1 Absolute mononuclear and heterophile leukocyte counts of 18 rabbits following the intravenous injection of 10 ml autogenous labeled lymphocytes suspensions and of 4 rabbits following the injection of 5 ml pyrogen-free isotonic sodium chloride.

stimulate sufficient fluorescence. An approximation of the rate of disappearance is given by the following figures: 98% of the animals had labeled lymphocytes in the peripheral circulation at the end of one hour, 72% at the end of two hours, and 42% at the end of three hours. Observations of blood drops revealed that in a given animal, the majority of transfused lymphocytes were removed from the blood during the first 90 minutes and, after this time, the concentrated buffy coat preparations had to be used. Attempts to find labeled cells in the blood were usually discontinued during the 4th hour because

they became so few in number. All of the labeled cells observed in the peripheral blood during the first three hours of an experiment were lymphocytes. Both small and medium sized lymphocytes were seen during the first hours, but medium sized lymphocytes were rarely found during the last two hours. The brightness of the label on the lymphocytes progressively decreased until it reached a constant, rather dim level after two hours.

In order to learn where the labeled lymphocytes were going after leaving the circulation, 8 rabbits were killed *two to three hours* after the transfusion, and frozen-dried sections of thymus, mesenteric lymph node, appendix, Peyer's patch, kidney, lung, muscle, liver, spleen, femoral bone marrow, and rib marrow were made. Cells with labeled nuclei, which with phase optics could be identified as lymphocytes, were found in the sections of bone marrow and lymphatic tissue, including the thymus (figs. 2–9) but not in other organs. In bone marrow, all sizes of labeled lymphocytes were regularly found. The great majority were enmeshed in the stroma and did not occur in the blood sinuses. In lymphatic tissue, large labeled lymphocytes were not frequently present and most of the labeled cells observed were small or medium lymphocytes. The labeled lymphocytes were in both the dense cortex and loose medulla in the lymphatic tissue. Labeled cell types other than lymphocytes have not been observed in either bone marrow or lymphatic tissue two hours after the injection of lymphocyte suspensions.

To follow further the fate of the lymphocytes in bone marrow and lymphatic tissue, 7 rabbits were killed *twelve hours* after the labeled lymphocyte transfusions and the same tissues were studied as in the two-hour group. In the lymphatic tissue, there were only a few differences between the two-hour and twelve-hour animals. The labeled lymphocytes were probably as numerous at 12 hours as at two hours, but the intensity of the label was decreased at 12 hours. In addition, on three or 4 occasions, pairs of labeled lymphocytes suggesting recent mitosis of the labeled cells were seen in the lymphatic tissue

from the 12-hour animals. None of the labeled lymphocytes showed any signs of degeneration. In bone marrow, labeled lymphocytes were only occasionally found 12 hours after the injections and most of the labeled cells could be identified by phase microscopy as myelocytes (figs. 10–13). These granulocytic cells were most often in small nests of granulocytopoiesis, usually a single labeled myelocyte among several unlabeled myelocytes. The labeled myelocytes varied from young cells with relatively large oval nuclei containing prominent nucleoli and a few granules in the cytoplasm, to more mature forms with smaller, more elongated and denser nuclei and many cytoplasmic granules. Labeled erythroid precursors were not observed. In contrast to the two-hour group, the rabbits killed at 12 hours also had labeled lymphocytes in the lamina propria and submucosa of the intestinal tract. Such cells were exceedingly rare and no more than 6 or 7 have been seen. Also, on just one occasion, a labeled lymphocyte was found as a free cell in the loose connective tissue of the neck region.

The question arises as to whether the labeled cells observed in bone marrow, lymphatic tissue, intestinal mucosa, and other connective tissue can account quantitatively for the nearly forty million labeled lymphocytes that were injected. On the basis of the figures given by Nye ('31) and Hellman ('14) it can be calculated that bone marrow and lymphatic tissue comprise approximately 3% of the total body weight of a rabbit, or 75 gm of the 2.5 kg rabbits used in this study. The 75 gm of tissue occupy approximately 75 cm^3 and, since the sections studied were about $5\,\mu \times 2\,mm \times 2\,mm$, each section comprised about 1 : 3,750,000 of the total bone marrow and lymphatic tissue. If one were to assume that all of the labeled lymphocytes were going to bone marrow and lymphatic tissue, than 3,750,000 sections should contain the 40,000,000 labeled leukocytes, or each section should have between 10 or 11 labeled cells. However, it is known that some of the labeled lymphocytes went to the lamina propria and submucosa of the intestine and connective tissue elsewhere in the body. It is also probable that some of the transfused cells were in a con-

dition unacceptable to the organism. In addition, Drinker and Yoffey ('41) have pointed out that Hellman's ('14) figure for the lymphatic tissue in the rabbit is an underestimation since it does not include the thymus and small lymphatic nodules scattered throughout the body. Inasmuch as labeled lymphocytes were seen in all lymphatic tissue studied, including the thymus, the transfused labeled cells must have been distributed over a greater bulk of tissue than the 75 gm used in the calculation. It follows, therefore, that each section of bone marrow and lymphatic tissue should actually contain fewer than 10 or 11 labeled cells. In reality, two or three were regularly seen in each section, but more labeled cells were probably present. Localization of more than two or three labeled lymphocytes or myelocytes in a given section is difficult because the labels become too dim as a result of the fading of diaminoacridine fluorescence when frozen-dried sections are stimulated by near ultraviolet light (De Bruyn et al., '50). The labels on the leukocytes were usually badly faded during the time required to find and identify two or three fluorescent cells. Taking these factors into consideration, it is probable that the majority of the labeled lymphocytes injected into the animal can be accounted for in the tissues either two hours or 12 hours after the transfusion.

The results obtained from the study of rabbits after autogenous labeled lymphocyte transfusions are meaningful only if they are not caused by the inadvertent labeling of cells present in the animal before the injection. Also, doubt would be cast upon the results if the same observations could be made after transfusions into rabbits of lymphocytes from another animal species. It is not reasonable to expect that foreign lymphocytes can be utilized by a rabbit in the same manner as autogenously transfused cells, and labeled lymphocytes or myelocytes seen in the tissues two or 12 hours after such an injection would probably indicate a transfer of the label during the experiment. Accordingly, to test the validity of the autogenous transfusion experiments, 15 rabbits were injected with labeled guinea pig lymphocytes. The lymphocytes were

obtained from the cervical lymph nodes and thymus and the suspensions were made in the same way as described for the transfusions of labeled rabbit cells. Eight rabbits were killed two hours, and 7 twelve hours, after the injections of the labeled guinea pig cells. The same tissues were taken as those used in studying the rabbits given rabbit lymphocytes. Cells with labeled nuclei were not seen in any of the tissues taken from any of the rabbits after the injection of labeled guinea pig lymphocytes.

DISCUSSION

The labeled lymphocytes seen in the tissues following the transfusion of labeled autogenous lymphocytes were actually cells from the original suspension and were not the result of (a) direct staining of tissue cells with the free dye in the labeled lymphocyte suspensions, or (b) the transfer of the label from one cell to another in the tissues of the animal. That the cells in the homopoietic tissues were not directly stained with the free dye is shown by the fact that such staining did not occur when the guinea pig cells were transfused into rabbits under conditions similar to the autogenous transfusions. A transfer of the label by phagocytosis of labeled cells can also be excluded. Such a transfer would involve the intracellular release of the dye and the subsequent secondary staining of the nucleus of the phagocyte with the dye thus released. This would result in the labeling of macrophages but cells of this type have not been observed with labeled nuclei following either autogenous or heterogeneous lymphocyte transfusions. The possibility that the dye content of an injected lymphocyte is released, either spontaneously or as a result of disintegration, to the surrounding tissue fluid and then reconcentrated in the nuclei of lymphocytes and myelocytes can also be rejected. The nuclei of blood cells have a relatively low affinity for diaminoacridines (De Bruyn et al., '50) and if the label were to transfer from a lymphocyte to another cell, it is reasonable to assume that at least some of the dye would be taken up by one or more of the many cell

types with a greater affinity for diaminoacridines than lymphocytes. Reticular cells, for example, have a high affinity for these dyes but these cells have not been seen with a label after a lymphocyte injection.

On the basis of the quantitative calculation presented earlier in this report, it appears that bone marrow and lymphatic tissue are two of the major sites of removal of lymphocytes from the circulation after an autotransfusion. The fact that lymphocytes leave the peripheral blood while passing through bone marrow agrees with the data of Yoffey and Parnell ('44). What happens to the lymphocytes in bone marrow and lymphatic tissue seems clarified by the results of the animals studied 12 hours after a labeled lymphocyte transfusion. At the 12-hour period most of the labeled cells in bone marrow were myelocytes. The question might arise as to whether the labeled myelocytes seen in the bone marrow 12 hours after the lymphocyte transfusion could not have been present in the initial suspension. This is not the case because myelocytes did not occur in the suspensions and labeled myelocytes were not in the tissue sections two hours after the transfusion. Since the evidence indicates the labeled myelocytes were not the result of some form of secondary staining and since the labeled myelocytes were not present in bone marrow two hours after the injection of the cell suspension, it seems highly probable that the labeled myelocytes were derived from the labeled lymphocytes known to be present in bone marrow shortly after the labeled lymphocyte transfusion. Under the conditions of these experiments, and at the time intervals studied following the injection, labeled erythroid precursors have not been observed in bone marrow. Such negative evidence does not eliminate the possibility that this transformation can take place under other conditions.

The fate of the lymphocytes that leave the blood to enter lymphatic tissue cannot be definitely ascertained from these data, but the available evidence tends to indicate that lymphocytes return to lymphatic tissue to reproduce their kind or to re-enter the blood via the lymphatics. Although actual mitotic

activity has not been observed in labeled lymphocytes in these tissues, pairs of labeled lymphocytes suggesting recent mitosis have been seen on several occasions. Furthermore, labeled lymphocytes in the lymphatic tissue did not show signs of degeneration either two hours or 12 hours after the injection and, therefore, it seems improbable that they ultimately degenerate in the lymphatic tissue.

Yoffey and Drinker ('39) have investigated the possibility of a return of lymphocytes to lymph nodes via the afferent peripheral lymphatics but they could not substantiate this hypothesis. The present work indicates that there is a large scale return of lymphocytes to lymphatic tissue via the blood stream. Schulze ('25) has observed lymphocytes in the walls of small blood vessels of lymph nodes and he believes that these cells are leaving the lymph node to enter the blood stream. It may be that many of these cells are leaving the circulation to enter lymphatic tissue.

The present study partially confirms the results of Bunting and Huston ('21) in that at least some of the circulating lymphocytes migrate to the intestinal wall. However, it could not be determined whether any of these cells actually leave the animal via this route since the amount of fluorescent debris in intestinal contents made it impossible to search for labeled lymphocytes within the lumen of the intestine. Only very few labeled lymphocytes were observed in the intestinal wall and even if these cells actually leave the organism this mechanism of removal of lymphocytes from the blood still accounts for only a relatively few of the disappearing lymphocytes as compared to bone marrow and lymphatic tissue.

These experiments do not eliminate the possibility that other mechanisms could contribute to the disappearance of the lymphocytes from the circulating blood. As mentioned before, the calculations and quantitative methods used are, of necessity, rather crude and it is possible that all of the labeled lymphocytes injected are not accounted for by those observed in lymphatic tissue, bone marrow, intestinal mucosa, and connective tissue of other organs. It may be that some of the

forty million cells injected, whether healthy or damaged by the technical procedures, are phagocytized by the macrophage system, although the present experiments have not produced evidence to substantiate this view. Also, it is possible that a rapid disintegration of labeled cells in the peripheral blood could occur without being detected.

There are a number of papers dealing with the disappearance from the circulation and subsequent fate of transfused leukocytes which in some aspects are at variance with the results presented here (Faludi, '38; Erf, '40; Weisberger et al., '49; Weisberger et al., '50; and Hammond and Novak, '50). In these papers, the conclusions relevant to the present study are largely based on the leukopenia which these workers describe as occurring immediately after the transfusion of large numbers of leukocytes. Caution should be exercised in basing conclusions on blood counts with respect to the removal of leukocytes from the circulation after intravenous injections, because it has been shown (Farr and LeQuire, '50), that the presence of varying amounts of pyrogenic material alone can produce leukocyte responses similar to those described by these authors. As previously noted, no leukopenia occurs after the injection of lymphocyte suspensions obtained from popliteal lymph nodes if a pyrogen-free technique is employed (fig. 1). In addition, some of these authors (Faludi, '38; Weisberger et al., '49; and Weisberger et al., '50) used leukocytes obtained from peritoneal exudates. It is questionable whether these exudate leukocytes can be considered the equivalent of leukocytes obtained from sources not involved in an acute inflammatory reaction. Another possible reason for some discrepancies is the use of homogeneous transfusions instead of autogenous transfusions (Faludi, '38; Weisberger et al., '50; Hammond and Novak, '50; Osogoe, '50). As a result of experiments in which they gave homogeneous transfusions of peritoneal exudate leukocytes labeled with P_{32}, Weisberger et al. ('50) conclude that "under the conditions of these experiments, the lungs play the major role in the prompt removal of

transfused leukocytes from the peripheral blood." It is apparent from their paper that they are dealing primarily with granulocytes and they make no specific statement as to the fate of the lymphocytes. Osogoe ('50) gave rabbits transfusions of homogeneous lymph node suspensions and reported finding many of the transfused lymphocytes in the lungs for the first 24 hours with the subsequent migration of these cells to the liver and spleen. In the present study, dealing with autogenous transfusion, no evidence was found in sections of lung or liver to indicate that autogeneous lymphocytes were removed from the circulation in these organs.

The results of these experiments with autogenously transfused labeled lymphocytes indicate that a major part of them go to the lymphatic tissue and bone marrow and, also, but to a minor extent, to the intestinal mucosa and connective tissue elsewhere. In bone marrow, the labeled lymphocytes become myelocytes. In lymphatic tissue, the evidence is less clear but indications are that lymphocytes return to lymphatic tissue via the blood where they may multiply. These findings are in agreement with the concept of the pluripotentiality of the lymphocyte as advocated by Dominici ('01), Maximow ('09), Weidenreich ('11), Dantschakoff ('16), Jolly ('23), Latta ('24), Lang ('26), and Bloom ('37).

It is realized that these results with labeled autogenous lymphocyte transfusions may not necessarily be an explanation for the fate of the lymphocyte in the intact animal. However, by inference, a basis is furnished for the hypothesis that similar events occur under physiologic conditions.

SUMMARY

The fate of the lymphocyte in the peripheral blood of rabbits has been studied by means of autogenous transfusions of labeled lymphocytes. The lymphocytes were obtained from the popliteal lymph nodes and their nuclei labeled with 3,6,diamino-10-methylacridinium chloride, a non-toxic vital nuclear stain which fluoresces when stimulated with near ultraviolet light.

Following the intravenous injection of labeled lymph node suspensions, most of the labeled lymphocytes were removed from the circulation during the first 90 minutes but a few remained in the peripheral blood for more than three hours in some of the animals. Rabbits were killed two and 12 hours after the injections and sections were prepared by the Altmann-Gersh freezing-drying technique. After two hours, lymphocytes with labeled nuclei were found in the bone marrow and lymphatic tissue, including the thymus, mesenteric lymph node, appendix, Peyer's patches, and white pulp of the spleen. At 12 hours, labeled lymphocytes were still present in lymphatic tissue, but at this time most of the labeled lymphocytes in bone marrow apparently had become myelocytes. Also at 12 hours, an occasional labeled lymphocyte was observed in the lamina propria and submucosa of the intestine and in connective tissue elsewhere in the body. Other organs did not contain cells with labeled nuclei at either time interval. Labeled cells of any type were not seen in the tissues of rabbits either two or 12 hours after the injection of labeled guinea pig lymphocytes.

On the basis of these experiments, it is suggested that the majority of the lymphocytes which leave the circulating blood go directly from the blood stream to (a) the lymphatic tissue where they may give rise to other lymphocytes, and to (b) the bone marrow where they can at least transform into cells of the granulocytic series.

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PLATE

EXPLANATION OF PLATE

The photographs of the fluorescent nuclei were taken with a Spencer 4 mm achromatic objective and a Zeiss 6 × compensating ocular. The phase photographs were taken with a Spencer 4 mm dark medium contrast objective and a Zeiss 6 × compensating ocular. Magnification in all figures is 900 ×.

PLATE 1

EXPLANATION OF FIGURES

2 Fluorescent nucleus of a medium-sized lymphocte in rabbit bone marrow two hours after the injection of labeled autogenous lymphocytes.

3 The same medium-sized lymphocyte shown in figure 2 as seen with phase microscopy.

4 Fluorescent nucleus of a medium-sized lymphocyte in rabbit bone marrow three hours after the injection of labeled autogenous lymphocytes.

5 The same medium-sized lymphocyte shown in figure 4 as seen with phase microscopy.

6 Fluorescent nucleus of a small lymphocyte in rabbit mesenteric lymph node two hours after the injection of labeled autogenous lymphocytes.

7 The same small lymphocyte shown in figure 6 as seen with phase microscopy.

8 Fluorescent nucleus of a small lymphocyte in rabbit appendix two hours after the injection of labeled autogenous lymphocytes.

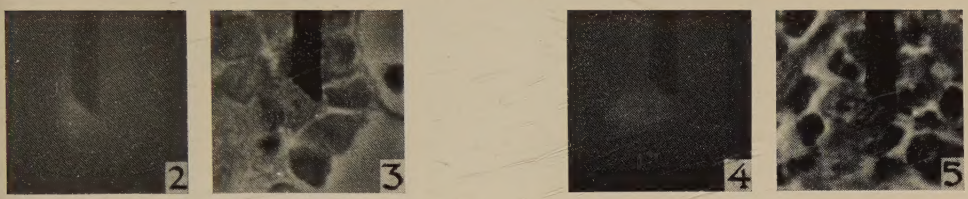
9 The same small lymphocyte shown in figure 8 as seen with phase microscopy.

10 Fluorescent nucleus of a myelocyte in rabbit bone marrow 12 hours after the injection of labeled autogenous lymphocytes.

11 The same myelocyte shown in figure 10 as seen with phase microscopy.

12 Fluorescent nucleus of a myelocyte in rabbit bone marrow 12 hours after the injection of labeled autogenous lymphocytes.

13 The same myelocyte shown in figure 12 as seen with phase microscopy.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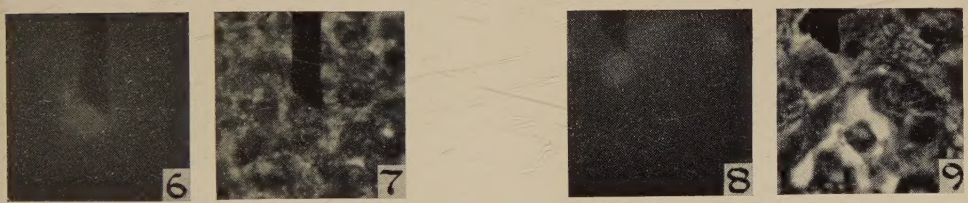
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